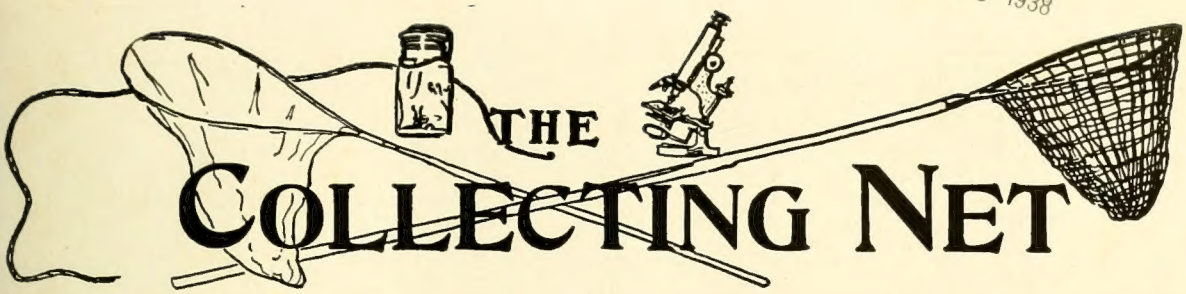

Vol. XIII, No. 1 SATURDAY, JULY 16, 1938 Annual Subscription, $1.50 Single Copies, 30 Cents.

LECTURE AND RESEARCH PROGRAM OF THE BIOLOGICAL LABORATORY AT COLD SPRING HARBOR

DR. ERIC PONDER
Director, The Biological Laboratory, Cold Spring Harbor

The summer activities of The Biological Laboratory of the Long Island Biological Association at Cold Spring Harbor began on June 22nd with the first meeting of the sixth of the Cold Spring Harbor Symposia on Quantitative Biology. The subject this year is that of Protein Chemistry, and at the time of writing twenty-two out of a total of thirty-five papers have already been read. The number of papers presented this year is considerably smaller than that presented in 1937; the attendance at the Symposium, however, has been larger, partly because those taking part have been in residence for a greater portion of the time, and partly because there has been a large number of visitors interested in the subjects of the papers.

As part of its policy of fostering a closer relation between biology and the basic sciences, the Laboratory invites each summer a group actively interested in a specific aspect of quantitative biology, or (*Continued on page* 4)

M. B. L. Calendar

TUESDAY, July 19, 8:00 P. M.

Seminar: Dr. K. C. Fisher and R. Öhnell: "The steady state frequency of the embryo fish heart at different cyanide concentrations."

Dr. Lena A. Lewis: "Studies of the refractory state resulting from repeated injections of cortical extract."

Dr. Emil Bozler: "Action potentials of visceral smooth muscles."

Dr. L. Irving: "Rhythmical changes in blood flow through muscles."

FRIDAY, July 22, 8:00 P. M.

Lecture: Dr. Oscar E. Schotté: Title to be announced later.

SYNTHESIS OF LIVING SUBSTANCE AS EXEMPLIFIED IN "CHILOMONAS PARAMECIUM"

S. O. MAST
Professor of Zoology, The Johns Hopkins University

I have been interested for many years in the problem of motor response and locomotion in invertebrates, especially Amoeba. Some time ago I began investigations on the transformation of energy and other processes, and the structures associated with locomotion and motor response in this form. All these characteristics have their origin in the food consumed. *Amoeba proteus*, the species used in this work, feeds extensively on *Chilomonas paramecium*. I therefore took up the study of the culture, the structure, and the chemical composition of this form. In this study Dr. D. M. Pace has been continuously associated with me.

Chilomonas is a minute flagellate barely visible to the naked eye. It is peculiar in that it contains large amounts of starch and fat. It has been cultured since 1912 in a solution of known composition, containing glycine acetate and inorganic salts. In this solution it obtains the nitrogen used in the synthesis of pro-

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WOODS HOLE IN HALF-TONE

Top row: Professor and Mrs. Thomas Hume Bissonnette and their young son in front of Hubbard House; Dr. D. E. Lancefield, associate professor of zoology, Columbia University; The Marine Biological Laboratory as seen from across the Eel Pond. **Middle Row:** The main entrance of the Marine Biological Laboratory; Players in the M. B. L. Tennis Tournament; Above: Glass blower Graham; Below: George M. Gray examines the specimens placed on display in the M. B. L. Lobby by the students of the invertebrate class. **Bottom Row:** Mrs. Frank Co Tui on the Mess Hall porch (she was unaware her picture was being taken); Dr. E. Uhlenhuth meditates; Marine life in the Fisheries' pool—why there are always onlookers around the pool.

(Large photographs were taken by Dr. Tuillio Terni of the University of Padua Medical College, Italy; others are staff pictures taken with a Zeiss Ikon Super Ikomat).

toplasm from the glycine and the carbon mainly from the acetate.

The green plants produce protoplasm from inorganic compounds, obtaining nitrogen from nitrates or ammonia, carbon from carbon dioxide, and energy from light. The animals and colorless plants, with the exception of a few bacteria, obtain nitrogen, carbon, and energy from complex organic compounds that are synthesized by the green plants.

We began our experiments by attempting to ascertain whether *Chilomonas* can obtain its nitrogen from simpler compounds than glycine and its carbon from simpler carbons than acetates. We found that it can obtain its nitrogen from ammonia and its carbon from carbon dioxide if the concentration of the carbon dioxide in the air is greatly increased, and if silicon is added to the culture solution. (The optimum concentration of carbon dioxide is fifteen percent.) Under these conditions *Chilomonas,* which has no chlorophyll, synthesizes protoplasm, proteins, starch, fat, and other complex compounds from inorganic compounds. No light is required. Silcon is not needed if acetate is present, and only a trace is needed if it is not present. It, therefore, is not a constituent of protoplasm in *Chilomonas.* It probably acts as a catalyst in the synthesis of starch from carbon dioxide and water.

The amount of substance that can be synthetized by *Chilomonas* is theoretically prodigious. For example, in one of our experiments, which was started with a single individual and continued for twenty-five days, if all the individuals had been kept and cultured, there would have been at the end of the experiment, 1.09×10^{26} individuals, and the total volume of these would have been, roughly, 612 billion cubic meters, which is enough to cover the whole state of Massachusetts with a layer approximately ninety feet thick. The starch in this mass would have been approximately nineteen billion cubic meters and the fat six billion cubic meters. This is sufficient to supply every individual in the United States with some seventy tons of starch and twenty tons of fat. As yet we have not, however, been successful in performing this experiment!

The culture solutions used in these experiments contain eight elements, carbon, hydrogen, nitrogen, oxygen, potassium, magnesium, sulphur, and phosphorus. But some of the compounds that contained these elements, as shown by spectrographic analysis, contained traces of other elements, e.g., the magnesium salt contained traces of calcium. We were unable, therefore, to ascertain whether or not *Chilomonas* can actually synthesize the substances found in it from these eight elements.

Calcium can be substituted for magnesium, but all calcium salts contain traces of magnesium, so we do not know whether this organism can synthesize without magnesium. We do know, however, that the rate of reproduction is higher when both magnesium and calcium are present in optimum concentration than when either is present without the other.

If calcium and magnesium are omitted the synthesis of starch and growth continue, the nucleus divides, but the cytoplasm does not divide, so that multinucleate monsters are produced. The function of these two elements seems to concern division of the cytoplasm.

Sulphur can probably be used in any form, organic or inorganic, completely oxidized or completely reduced, but the rate of reproduction varies greatly with the concentration of the sulphur and the optimum concentration varies greatly with the form of the sulphur. The rate of reproduction is not, however, specifically related to the degree of oxidation. If sulphur is omitted there is a marked accumulation of fat, i.e., there appears to be fatty degeneration which eventually results in death. The absence of sulphur does not interfere with growth or the synthesis of starch, but it apparently interferes with the oxidation of fat, resulting in its accumulation. This conclusion is supported by the fact that in the absence of sulphur the rate of consumption of oxygen decreases.

Robertson (1921) found in species of the cilliate, *Enchelis,* that the rate of reproduction varies directly with the density of population. Later he found that the same is true in another cilliate, *Colpidium.* He concluded that probably all cells produce growth promoting substance. This conclusion is supported by the results obtained in observations on similar organisms by three other investigators, but it is opposed by those obtained by twelve others. The variation in results obtained by these different investigators is usually assumed to be correlated with differences in the kind, the quantity, or the availability of the food present in the solutions used by them. Robertson's conclusion has therefore been practically abandoned.

Chilomonas paramecium is a very favorable organism for the investigation of this problem because the food can be accurately controlled. We ascertained the rate of reproduction in one series

THE COLLECTING NET has been entered as second-class matter July 11, 1935, at the Post Office at Woods Hole, Mass., under the Act of March 3, 1879. It is devoted to the scientific work at marine biological laboratories. It is published weekly for eight weeks between July 1 and September 15 from Woods Hole, and is printed at The Darwin Press, New Bedford, Mass. Its editorial offices are situated on Main Street, Woods Hole, Mass. Between June 1 and October 1 communications should be addressed to Woods Hole, Mass.; at other times they should be directed to THE COLLECTING NET, Garrison, N. Y. Single copies, 30c; subscription, $1.50.

of cultures in which the number of chilomonads put into a given volume of culture fluid varied, in another series in which the same number were put into different volumes, in another in which both the volume and the number varied and in still another in which different numbers were put into the same volume, left for different periods of time, then removed and others added (one per .025 cc.). The results obtained in these different series of solutions are essentially the same. They show that as the density of population increased the rate of reproduction increased to a maximum and then decreased to zero, and that the rate of reproduction increased more than fifty percent in some of the tests.

We demonstrated that the relation between density of population and rate of reproduction is not due to the action of mitogenetic rays, to contact stimulation, or to the consumption of compounds in the solution, but to the production of a substance, X, by the chilomonads.

If a solution which contains this substance in maximum concentration, i.e., a solution taken from an old culture, is subjected to a temperature of 100° C. for different periods of time and then tested for rate of reproduction of *Chilomonas* in it, the rate of reproduction increases from 0 to a maximum, as the time at 100° increases, and then decreases to that which obtains in fresh culture fluid. These results indicate that the substance which accelerates the rate of reproduction is the same as that which retards and inhibits it and that this substance is heat labile.

By putting a given number of chilomonads into a given amount of culture fluid and leaving them for definite periods of time, and then removing them and testing this fluid for rate of reproduction, we found that the lowest effective concentration of the substance, X, is produced with one chilomonad in 0.1 cc. culture fluid for four hours. If it is assumed that during this period a chilomonad produces substance X equal in volume to 0.1 of its own volume, the concentration would be approximately one part of X in 800 million parts of culture fluid. We found that, with seventy-five chilomonads in one cc. of culture fluid for four hours, the concentration of substance X is optimum and that on the basis of the preceding assumption this concentration would be approximately one part of X in ten million parts of culture fluid.

The fact that *Chilomonas paramecium* produces a growth-promoting substance does not prove that Robertson's conclusion is valid, but it does lend support to it, as does also the fact that such substances are produced by some cells in plants and probably also in some eggs.

If the production of a substance which in low concentration promotes and in high concentration retards or inhibits growth is wide-spread in cells, it is obviously of the greatest importance in all problems concerning growth (including cancer), reproduction and senescence, and doubtless has important bearing on many others.

(This article is based on a lecture given by Dr. Mast on July 8 at the Marine Biological Laboratory.)

LECTURE AND RESEARCH PROGRAM OF THE BIOLOGICAL LABORATORY

(Continued from Page 1)

in methods and theories applicable to it, to carry on their work and to take part in a symposium at the Laboratory. The aim is that every important aspect of a given subject should be adequately represented, from the physical and chemical, as well as from the biological point of view.

The program is printed below.

PROGRAM

Wednesday, June 22nd

R. Keith Cannan, New York University Medical College: Hydrogen ion dissociation curves of proteins.

D. C. Carpenter, New York State Agricultural Experiment Station, Geneva, N. Y.: The influence of neutral salts on the optical rotation of gelatin.

Thursday, June 23rd

E. J. Cohn, Harvard Medical School: Number and distribution of the electrically charged groups in proteins.

Friday, June 24th

Robert M. Herbst, College of Physicians and Surgeons, Columbia University: Some relationships between keto acids and amino acids.

Joseph S. Fruton, Rockefeller Institute for Medical Research, New York: Protein structure and proteolytic enzymes.

Saturday, June 25th

John T. Edsall, Harvard Medical School: Raman spectra and structure of amino acids and peptides.

Monday, June 27th

David I. Hitchcock, School of Medicine, Yale University: Proteins as amphoteric electrolytes.

Carl Niemann, California Institute of Technology: The chemistry of protein structure.

Tuesday, June 28th

H. B. Vickery, Connecticut Agricultural Experiment Station, New Haven, Conn.: Metabolism of proteins in green leaves.

Wednesday, June 29th

Henry B. Bull, Northwestern University Medical School: Protein denaturation.

Alfred Mirsky, Rockefeller Institute for Medical Research, New York: Protein denaturation.

Thursday, June 30th

Dorothy Wrinch, Oxford University, England: Recent developments of the cyclol hypothesis of protein structure.

G. GRABAR, College of Physicians and Surgeons, Columbia University: The influence of collodion membrane structure on the ultrafiltration of proteins.

Friday, July 1st

IRVING LANGMUIR, General Electric Company Research Laboratory: Protein monolayers.

LAURENCE S. MOYER, University of Minnesota: Electrokinetic aspects of protein chemistry.

Saturday, July 2nd

HANS NEURATH, Cornell University: Diffusion measurements of proteins: serum albumin and tobacco mosaic virus.

Tuesday, July 5th

JOHN W. MEHL, Harvard Medical School: Double refraction of flow in protein solutions.

J. F. DANIELLI, University of London, England: Protein films at the oil-water interface.

Wednesday, July 6th

KURT G. STERN, School of Medicine, Yale University: The relationship between prosthetic group and protein carrier in certain enzymes and biological systems.

Thursday, July 7th

W. T. ASTBURY and FLORENCE O. BELL, University of Leeds, England: Some recent developments in the X-ray study of proteins and related structures.

HUGO FRICKE and EDWARD PARKER, The Biological Laboratory: The effect of X-rays on proteins.

Friday, July 8th

J. W. WILLIAMS, University of Wisconsin: The physical chemistry of the prolamines.

Saturday, July 9th

R. W. G. WYCKOFF, Lederle Laboratories: The ultracentrifugal study of viruses and other macromolecules.

Monday, July 11th

ABRAHAM WHITE, School of Medicine, Yale University: Protein hormones.

VINCENT du VIGNEAUD, George Washington Medical School: The role that insulin has played in our concept of protein hormones.

Tuesday, July 12th

RICHARD J. BLOCK, New York State Psychiatric Institute and Hospital, New York: Studies in comparative biochemistry of the proteins.

Wednesday, July 13th

JACINTO STEINHARDT, Harvard Medical School: Solubility anomalies in crystalline proteins.

W. M. STANLEY and H. S. LORING, Rockefeller Institute for Medical Research, Princeton, N. J.: Properties of virus proteins.

Thursday, July 14th

KARL MEYER, College of Physicians and Surgeons, Columbia University: The chemistry and biology of glycoproteins.

Friday, July 15th

ROGER HERRIOT, Rockefeller Institute for Medical Research, Princeton, N. J.: Crystalline pepsinogen and its conversion to pepsin.

Monday, July 18th

M. KUNITZ, Rockefeller Institute for Medical Research, Princeton, N. J.: Solubility curves of crystalline enzymes.

Tuesday, July 19th

MICHAEL HEIDELBERGER, College of Physicians and Surgeons, Columbia University: Protein constitution and immunological behavior.

FORREST E. KENDALL, New York City Department of Hospitals, Welfare Island: The use of immunochemical methods for differentiation and determination of human serum proteins.

Wednesday, July 20th

ELOISE JAMESON, School of Medicine, Stanford University: A phase rule study of the proteins of blood serum: variations in the principal protein complex.

Thursday, July 21st

SAMUEL GRAFF, College of Physicians and Surgeons, Columbia University, and L. G. BARTH, Columbia University: The composition of tissue proteins.

L. G. BARTH, Columbia University, and SAMUEL GRAFF, College of Physicians and Surgeons, Columbia University: The chemical nature of the amphibian organizer.

NOTE: Papers of these symposia, together with edited discussions, are published as COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY. The above papers and discussions will constitute Volume VI. Volume I (1933) dealing largely with surface phenomena, Volume II (1934) with some aspects of growth, Volume III (1935) with photochemistry and some applications to biology and medicine, Volume IV (1936) with excitation phenomena, and Volume V (1937) with internal secretions, may be purchased from the Biological Laboratory.

The course in Surgical Methods in Experimental Biology is again being given by Dr. G. W. Corner of the University of Rochester, and the course in Experimental Endocrinology by Dr. H. O. Haterius of Ohio State University and Dr. Robert Gaunt of New York University. The two courses work together, lectures being held in common, and the students in the endocrinology course working to some extent on animals prepared in the course in surgical methods. During the second part of the summer the Laboratory will offer courses in Marine and Fresh Water Zoology, given by Dr. H. T. Spieth of the College of the City of New York and Dr. W. A. Castle of Brown University, and in Plant Ecology, given by Dr. Stanley A. Cain of the University of Tennessee. Speaking generally, the registration is somewhat lower than it was last year, but this is probably attributable to the present depression.

For several years past the Laboratory has been supporting investigations in plant sociology, and it is clear that there is a need for correlation of the different branches of the field of community study. The Laboratory has accordingly invited a group of botanists and zoologists, interested in community problems, to present a series of papers during the week of August 29th. Not more than two papers will be read on any one day, an arrangement which allows ample time for the presentation of each subject and discussion by the members of the conference and by visitors, and a series of field trips will be arranged for during the period of the conference. There will be nine papers which, together with edited discussion, will be published as No. 1, Volume 20, of the American Midland Naturalist in January, 1939.

The following investigators are expected to take part: G. E. Macginitie, F. E. Eggleton, Stanley A. Cain, Henry S. Conrad, A. E. Emerson, H. A. Gleason, J. R. Carpenter, T. Lippman, and T. Park.

As was remarked last year, the policy of the Laboratory is to keep the Symposium small and to restrict participation to those who are experts in their field, but at the same time an obvious extension of the Symposium idea is to give investigators a chance to work with each other in the laboratory. Last year the Rockefeller Foundation increased the allocation for the Symposia in order to carry out an experiment along these lines, and it is gratifying to report that the result has been a great increase in the amount of independent research work which is being carried out this summer. In addition to the all-year-round work in Biophysics and Physiology, seventeen independent investigators are fully occupied in the comparatively small amount of research space which the Laboratory can put at the disposal of summer visitors. Dr. H. F. Blum, assisted by Mr. Chester Hyman, has been here since the beginning of June working on the kinetics of photodynamic hemolysis. A research group, consisting of Drs. H. O. Haterius, W. O. Nelson, Robert Gaunt, Hermann Rahn, Mr. M. J. Kempner, Mr. Charles Lloyd, and Miss Eleanor Loomis and Miss Arline Fox, are carrying out investigations on various endocrine problems, most of which were raised as questions in last year's Symposium. Dr. Harold A. Abramson, assisted by Dr. Janet Daniel, Miss Cecile Metz, Miss Jamie Porter and Miss Lora Jennings, is continuing his investigations on electro-kinetics and the penetration of drugs into the skin. Dr. F. R. Steggerda is investigating potential of frog skin, and Dr. V. E. Morgan is developing a method for the preparation of myoglobin; Dr. Hugh Davson is spending the entire summer at the Laboratory and continuing experiments on permeability of red and white cells to various ions, and Dr. J. F. Danielli has been measuring properties of surface films, principally in connection with the effect of photodynamic processes upon them. Dr. Hugo Fricke, assisted by Dr. Edward Parker, is engaged in developing the effect of irradiation upon solutions of proteins, his work on surface conductance and dipole moments being temporarily interrupted until the return of Dr. Adolph Parts from Estonia. The writer has little time for research work during the Symposium period, but the program for the remainder of the summer calls for investigation of various aspects of intravascular hemolysis and a study of the effect of storage of whole blood under the conditions now being used in the so-called "Blood Banks."

EXPERIMENTAL STUDIES ON THE REPRODUCTIVE SYSTEM OF THE MALE ANURAN

Dr. Roberts Rugh

Department of Zoology, Hunter College

Several years ago a study of the morphology and physiology of the female frog was presented here. It seemed appropriate that a similar study be made of the male frog. Some of the data from this study is to be presented at this time.

The material of this paper is taken largely from work on the leopard frog, *Rana pipiens* and the Spring Peeper, *Hyla crucifer*. The testis of the anuran is joined to the kidney by the mesorchium within which are 8-12 branching vasa efferentia. There is considerable variation in size, number, and arrangement of these ducts (even on the two sides of the same animal) and a few of them end blindly within the mesorchium. By the injection of a solution (made up of one part India Ink and two parts Holtfreter's modification of Amphibian Ringer's) into the vas in the direction of the testis, the arrangement of the seminiferous tubules can be made out. A single vas is seen to be related to many such tubules, some of them bifurcating at the periphery of the testis. There is no difficulty in identifying the vas efferens as opposed to the nearby blood vessels. If the vas is injected with the above solution, in the direction of the kidney, and the course of the ink is observed under the binocular microscope, the path of spermatozoa through the kidney can be determined. The vas efferens, as it enters the kidney near (but not at) the mesial margin, immediately branches and feeds a number of malphigian corpuscles. The 8-12 vasa efferentia feed approximately 40-50 malphigian corpuscles, all located within the anterior two-fifths of the kidney. The malphigian corpuscles and the contributing branches of the vasa efferentia are placed ventrally within the kidney near its mesial margin. The ink is seen to leave the malphigian corpuscle and pass dorsally through the kidney substance and then directly across the kidney to the ureter (Wolffian or mesonephric duct). None of the ink is seen to pass posteriorly along the mesial margin of the kidney as would be the case if Bidder's canal were present.

Errors in interpretation of the effect of hormones on the male gonads have been made because of faulty manipulation. The testis of the hibernating frog contains mature spermatozoa which can be liberated into the seminiferous tubules (and subsequently into all parts of the genital tract) by mechanical pressure on the testis.

Only the slightest pressure on the testis is necessary to show masses of spermatozoa passing through the vasa efferentia, into the kidney, and across the kidney into the ureter. This can be easily demonstrated under the binocular microscope in the living organs. For this reason it is very important that in studying the effects of hormones, etc., on the testis, excision of the gonad must be rapid and with little manipulation, and fixation must be immediate. Unlike the testis of many other forms, the anuran testis contains masses of gametes at the identical stage of development.

The action of the anterior pituitary hormone on the anuran ovary has been demonstrated. This hormone, when injected into the body cavity of a hibernating male anuran, has a similar effect on its testes. It causes the liberation of spermatozoa into the seminiferous tubules, and the genital tract. In practice it has been found that the peritoneal injection of the hormone has elicited a quicker response than has subcutaneous implantation of the gland. This is possibly due to the fact that on the ventral face of the kidney there are 200-250 peritoneal funnels (erroneously called nephrostomes) in *Rana pipiens* (22-35 in *Hyla crucifer*) which carry coelomic contents directly into the venous system.

If the left testis of a hibernating frog is excised and studied, all of the spermatozoa will be seen clustered about their Sertoli cells, awaiting liberation (probably by the host's anterior pitiuitary hormone) during the normal breeding season. If the frog is then treated with the anterior pituitary hormone and its remaining (right) testis is examined in 24 hours, all of the spermatozoa in that testis will be seen to be liberated into lumina of the seminiferous tubules and within 36 hours the tubules will be practically emptied, except for spermatogonia retained for development the following season. As many as 28 spermatozoa have been seen clustered about a single Sertoli cell.

It is entirely possible that the spermatozoa are liberated from their tubules in a manner similar to the liberation of the anuran egg from its follicle, i.e., by the muscular contraction of the surrounding tissues. After liberation, it is very probable that the spermatozoa become motile, at least until they get into the slightly acid excretory fluids of the uriniferous tubules. The longitudinal collecting tubes within the testes carry the spermatozoa to the vasa efferentia and sections of the related kidney will show them clustered about the glomeruli of malphigian corpuscles and passing out through related uriniferous tubules to the ureter. The path of spermatozoa, histologically determined, is correlated exactly with the path as determined by the micro-injection of ink into the vasa efferentia.

The major results of this investigation may thus be summarised: First, the anterior pituitary hormone not only stimulates sexual behavior of the male (amplexus) but in a way similar to its effect on the anuran ovary it liberates mature spermatozoa from their Sertoli cells into the related genital tract. Second, the genital tract (as determined by micro-injection and the histological study of sexually activated males) consists of the seminiferous tubules, longitudinal collecting tubules, vasa efferentia, malphigian corpuscles, uriniferous tubules, and the seminal vesicle. There is neither morphological nor histological evidence of a Bidder's canal, nor is such a canal necessary to the normal functioning of the genital system of the male anuran.

Editorial Note: The injected and the histological preparations shown on lantern slides at the evening meeting are on hand and Dr. Rugh will be glad to show them to anyone interested.

(This article is based on a seminar report given at the Marine Biological Laboratory on July 5).

SPECIAL LECTURES BEFORE THE EMBRYOLOGY CLASS

The following is the list of special lectures which have been given or have so far been scheduled to be given before the Embryology Class. One or two lectures may be added to this list:

June 25—GEORGES VANDEBROEK, Morphogenetic movements during the gastrulation of Scyllium.

July 6—DR. CHARLES PACKARD, Topics from the history of embryology.

July 8—DR. B. H. WILLIER, Hormonal control of sex-differentiation.

July 13—DR. T. H. MORGAN, Genetics and embryology.

July 20—DR. PAUL WEISS, The colloidal-physical basis of some developmental patterns.

July 22—DR. E. R. CLARK. Developmental processes in the adult.

July 27—DR. E. G. CONKLIN.

Two changes have been made in the staff of the course in embryology since last summer. Dr. Douglas Whitaker of Stanford University has taken the sections of the course formerly given by Dr. Charles Packard and which deal with the Crustacea, fertilization of Nereis and with cell lineage. Dr. W. W. Ballard of Dartmouth College is in charge of the work formerly given by Dr. L. G. Barth on the Coelenterates and the Tunicates.

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris Gorokhoff, Bradford Chambers, and Garnette McClure.

Entered as second-class matter, July 11, 1935, at the U. S. Post Office at Woods Hole, Massachusetts, under the Act of March 3, 1879.

BIOLOGISTS AND "THE COLLECTING NET"

For twelve summers The Collecting Net has reported upon the activities of the Marine Biological Laboratory and more recently upon those of other marine stations as well. Its volumes form a valuable record of the work and people of the laboratory. In its thirteenth year the journal looks forward to the ever increasing support of marine biologists. The growing subscription list indicates an interesting trend; the number of "alumni of the M. B. L." who subscribe, together with the many institutional libraries, has brought up the "outside" mailing list to a point almost as high as the Woods Hole group.

The number of people who take part in contributing directly or indirectly to each issue is surprisingly large. For example thirty-two individuals at the Woods Hole laboratories gave of their services in some way to the editorial content of this particular issue; this figure excludes the many more who have co-operated in the preparation of the "Directory for 1938." Probably over two hundred biologists took part in the making of The Collecting Net last summer. For this help we are thankful; with the intimate interest of so many people the journal must continue to prosper.

Notes

We regret that the publication of the six-page directory in this issue has made it necessary to omit textual material as well as two pages of advertisements.

Eight issues of The Collecting Net will be published during the summer, the last appearing on September 3. Subscribers who leave Woods Hole before that time may have their copies forwarded to them without charge.

The office of The Collecting Net is more conveniently located this year than it has been in the past. It occupies space in a building on the main street about a block East of the Marine Biological Laboratory. We hope that many biologists will drop in with notes and news for our "Items of Interest" page.

Introducing

William Henry Newton, reader in physiology, University College, London; Rockefeller Foundation fellow at Yale University.

Born in Lincolnshire County, England, Dr. Newton attended Manchester University, receiving both M.Sc. and M.D. degrees there. He majored in physiology, writing his master's thesis on the effect of hydrogen ion concentrations on smooth muscle, and his doctor's thesis on certain aspects of uterine muscle. He was awarded the Platt Physiological Scholarship of the University of Manchester for part of that time.

After a term as house physician at the Manchester Royal Infirmary, he was appointed in 1930 to the Shapley Scholarship at University College, London. After some years as lecturer in Professor Lovatt Evans' physiology department there, he was promoted to the position of reader in physiology at the University of London.

Dr. Newton has written a number of articles in the field of physiology, dealing especially with various aspects of pregnancy in animals. He edited the last edition of Evans' "Recent Advances in Physiology," which appeared in 1936.

Dr. Newton came to America last August under a Rockefeller Foundation fellowship to work in endocrinology with Dr. Edgar Allen at Yale University, having first spent six weeks at Woods Hole. This summer he is studying pregnancy with reference to placenta, especially in connection with water metabolism.

Upon the conclusion of his summer's work, Dr. Newton will return to England to resume his duties at the University of London. He will sail from New York on September 9.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
July 16	7:32	7:48
July 17	8:11	8:32
July 18	8:51	9:21
July 19	9:40	10:11
July 20	10:25	11:04
July 21	11:25	
July 22	12:04	12:21
July 23	1:01	1:20

In each case the current changes approximately six hours later and runs from the Sound to the Bay.

ITEMS OF INTEREST

DR. CHARLES PACKARD, acting director of the Marine Biological Laboratory, will resign the position that he has held for seven years as Clerk of the Corporation. Members will elect his successor at their annual meeting on August 11.

DR. and MRS. FRANK BLAIR HANSON are visiting Woods Hole for the month of July; they are living in the Dormitory. Dr. Hanson is the Associate Director of the Division of Natural Science for the Rockefeller Foundation.

DR. G. VANDEBROEK, assistant in embryology at the University of Ghent is leaving for New Haven to-day, where he will work for a month at Yale University before returning to the Marine Biological Laboratory.

Rockefeller Foundation Fellows in the Natural Sciences at the Marine Biological Laboratory

Eleven investigators are carrying out research problems at the Marine Biological Laboratory with the assistance of grants from the Rockefeller Foundation. Three additional fellows (with an asterisk before their names in the list below) will visit the laboratory sometime during the summer.

SARAH C. BEDICHEK, Associate Professor of Genetics, North Texas Agricultural and Mechanical Arts College.

L. VON BERTALANFFY, Privat-Dozent, University of Vienna.

*J. Y. BOGUE, Lecturer in Physiology, Royal Veterinary College, London; Honorary Lecturer in Physiology, University College.

H. J. CURTIS, Instructor in Physiology, College of Physicians and Surgeons, Columbia University.

HELMUT GORDON, Assistant in Physiology, University of Budapest.

PIERRE GRABAR, Assistant Physical Chemist in Biology, Pasteur Institute.

PETER GRAY, Lecturer in Zoology, University of Edinburgh.

STEPHEN KARADY, Assistant in Internal Medicine, University Szeged, Hungary.

MANFRED KIESE, Assistant in Pharmacology, University of Berlin.

*KENNETH MATHER, Assistant Director of Genetics, The John Innes Horticultural Institution, London.

WM. H. NEWTON, Senior Lecturer in Physiology, University College, London.

J. STEINHARDT.

M. J. D. WHITE, Lecturer in Zoology, University College, London.

*J. M. YOFFEY, Senior Lecturer in Anatomy, University of South Wales, Cardiff.

The Physiology Class at the M. B. L. went on a trip to Oak Bluffs last Wednesday evening in the *Winifred*. Some thirty-five persons were present, including several persons that were invited by the class. The trip was paid for by the balance remaining from the annual class picnic.

NEW HUMOR AND NEUROHUMORS

On more than one occasion last night Dr. Parkers' jokes "brought down the house", as an amused physiologist expressed it. One listener's version of the lighter parts of his evening lecture follow:

After this lecture was announced I was accosted by a great many witty friends who made jokes and puns on the idea of what neurohumors were. There were in fact 11½ jokes. I list them as such because the one by Dr. Oliver Strong was so low! At his expense I shall take the liberty of telling it:

Many people appear to have rather strange ideas about neurohumors. The mother of a precocious child was entertaining guests in the living room. "My little daughter," she warned her guests, "has been seeking too much attention recently; if she should come into this room and act peculiarly, please let us pay no attention to her. We must ignore her completely." Soon muffled steps were heard on the stairs and into the living room walked the child, stark naked, her body all aglow. She walked through the room and out again. No one moved or commented. After the guests had departed the mother went to her daughter's room, and the child, now fully clothed, exclaimed radiantly: "It worked, Mother, it worked!" "What?" questioned the puzzled mother. "I took off my clothes, rubbed myself all over with vanishing cream, and sure enough, when I walked throug the living room, no one even saw me!"

Many persons' conception of neurohumors is quite similar to this child's ideas about vanishing cream.

Chromatophores are unique. In the higher vertebrates and in man there are practically none. We have in our skin some possibilities of blushing more or less, of which a last remnant still remains. I have already remarked in previous lectures that this is now a lost art. Generally, I have been led to observe the rather remarkable phemenon that a few are still able to blush and that it takes place on the face and neck. In my generation we had no idea how far it went, but we have in the young women of to-day a great opportunity for the study of the extent of this marvelous social gesture.

CRITERIA OF PLACENTAL ACTIVITY IN THE MOUSE

Dr. W. H. Newton
Visiting Research Assistant, Department of Anatomy, Yale University

Destruction of the foetuses in pregnant mice can be performed without disturbing the placentae, which are retained and delivered at normal full term. In the interval, body weight already gained is maintained and oestrous cycles are suppressed; delivery is followed by loss of weight and by oestrus as in normally pregnant animals. Complete evacuation of the uterus leads to a prompt loss in weight, and oestrus follows, showing that presence of the placentae is the significant factor. In collaboration with Dr. F. J. Lits, these findings have been extended to include development of the mammary glands and reabsorption of the symphysis pubis, which at the end of normal pregnancy is replaced by a ligament two or three millimetres in length. Both mammary development and public separation occur normally in the absence of foetuses, but in the presence of retained placentae.

Foetal destruction is performed as a routine on the 12th day of pregnancy. If this is accompanied or followed closely by oöphorectomy, the course of events is altered. Placentae tend to be aborted within about two days, but if retained they may remain for several days after the normal time for delivery. The body weight, though well maintained during the one or two day interval between foetal destruction and oöphorectomy, when this exists, falls abruptly after removal of the ovaries. Transformation of the symphysis pubis does not take place even though the placentae are retained. The almost complete simulation of pregnancy which occurs ordinarily after foetal destruction is, therefore, largely dependent on the simultaneous presence of the ovaries and placentae.

The mammary glands furnish an exception. If placental abortion occurs after oöphorectomy, they are found to have undergone, at the 18th day, profound regression from the 12-day condition. (In two cases in which placentae had been palpated in the uterus the day before autopsy, the density of the glands approximated that of 12-day glands, but involution was in progress). If living placentae are retained till the 18th day, no involution is found, and the degree of growth of the glands ranges from a maintenance of the 12-day condition to full-term development. Distension of ducts and alveoli shows the presence of secretion, but although the glands may be indistinguishable from those of the end of normal pregnancy when seen in the whole mount, histological section may reveal a comparative lack of vacuoles in the secreting cells. In this alone they are unlike glands developed in the presence of both retained placentae and ovaries.

It is possible that, just as many of the phenomena accompanying placental retention are dependent on the simultaneous presence of the ovaries, so mammary development is dependent on the simultaneous presence of, for instance, the hypophysis. Although it seems the most likely explanation, the placenta does not necessarily act on the ovary and the mammary gland (or hypophysis, etc.) by means of an internal secretion or secretions. Uterine distension has been eliminated by slitting the uterus, but afferent nervous stimuli resulting from the presence of the placenta are not excluded. A particular physiological state of the endometrium, which itself may produce an internal secretion, may also result from the continued attachment of the placenta.

A DEMONSTRATION OF THE RELEASE OF AN OXYTOCIC HORMONE FROM THE HYPOPHYSIS

Drs. J. K. W. Ferguson and H. O. Haterius
Dept. of Physiology, Ohio State University

By inserting bipolar electrodes into the brain of an anesthetized rabbit it is possible to apply electrical stimulation to the pituitary stalk. When this is done a few hours or days after parturition while recording the contractions of the uterus, a very striking increase in the frequency and amplitude of the contractions is observed in about two minutes. The augmentation in uterine motility resembles closely the effect of 0.3 units of pituitrin injected intravenously. It is entirely unlike the effect of epinephrine, which under these circumstances has a preponderantly inhibitory effect. Furthermore the effects of epinephrine wear off in a few minutes while the effects of electrical stimulation and of pituitrin last for nearly an hour.

The response to electrical stimulation of the stalk can be abolished by destruction of the stalk or of the posterior lobe of the pituitary complex, but cannot be abolished by severing all the nervous connections between the brain and the uterus.

In the light of these experiments there seems to be no need to doubt any longer that the pituitary complex is capable of secreting an oxytocic hormone. It seems clear, too, that the hormone must play an important part in parturition because after destruction of the posterior lobe the contractions of the uterus become progressively weaker and less frequent. It remains for further experiments to show how the secretion of the hormone is controlled in the normal animal.

(This article is based on a seminar report given at the Marine Biological Laboratory on July 5).

DIRECTORY FOR 1938

KEY

Laboratories		Residence	
Botany Building	Bot	Apartment	A
Brick Building	Br	Dormitory	D
Lecture Hall	L	Drew House	Dr
Main Room in Fisheries Laboratory	M	Fisheries Residence	F
Old Main Building	OM	Homestead	Ho
Rockefeller Bldg.	Rock	Hubbard	H
Supply Dept.	S	Kahler	Ka
		Kidder	K
		Whitman	W

MARINE BIOLOGICAL LABORATORY

THE STAFF

ZOOLOGY

Investigation

Calkins, G. N. prof. proto. Columbia.
Conklin, E. G. prof. zool. Princeton.
Grave, C. prof. zool. Washington.
Jennings, H. S. prof. zool. Hopkins.
Lillie, F. R. prof. emb. emer. Chicago.
Mast, S. O. prof. zool. Hopkins.
McClung, C. E. prof. zool. Pennsylvania.
Morgan, T. H. dir. biol. lab. California Inst. Tech.
Parker, G. H. prof. zool. Harvard.
Wilson, E. B. prof. zool. Columbia.
Woodruff, L. L. prof. proto. Yale.

Instruction

Bissonnette, T. H. prof. biol. Trinity. in charge.
Crowell, P. S., Jr. instr. zool. Miami.
Hadley, C. E. assoc. prof. biol. N. J. State Teachers'.
Kille, F. R. asst. prof. zool. Swarthmore.
Lucas, A. M. assoc. prof. zool. Iowa State.
Matthews, S. A. asst. prof. biol. Williams.
Nelsen, O. E. asst. prof. zool. Pennsylvania.
Rankin, J. S. fel. zool. Amherst.
Waterman, A. J. asst. prof. biol. Williams.

PROTOZOOLOGY

Investigation (See Zoology)

Instruction

Calkins, G. N. prof. proto. Columbia. in charge.
Hughes, Elizabeth D. lect. zool. Barnard.
Kidder, G. W. asst. prof. biol. Brown.

EMBRYOLOGY

Investigation (See Zoology)

Instruction

Ballard, W. W. asst. prof. biol. & anat. Dartmouth.
Goodrich, H. B. prof. biol. Wesleyan. in charge.
Hamburger, V. asst. prof. zool. Washington.
Schotté, O. asst. prof. biol. Amherst.
Whitaker, D. M. prof. zool. Stanford.

PHYSIOLOGY

Investigation

Amberson, W. R. prof. phys. Marvland Med.
Bradley, H. C. prof. phys. chem. Wisconsin.
Garrey, W. E. prof. phys. Vanderbilt Med.
Lillie, R. S. prof. gen. phys. Chicago.
Mathews, A. P. prof. biochem. Cincinnati.

Instruction

Chambers, R. prof. biol. New York.
Ferguson, J. K. W. asst. prof. phys. Ohio State.
Fisher, K. C. asst. prof. exper. biol. Toronto.
Höber, R. visit. prof. phys. Pennsylvania.
Irving, L. prof. biol. Swarthmore. in charge.
Prosser, C. L. asst. prof. phys. Clark.
Sichel, F. J. M. instr. phys. Vermont Med.

BOTANY

Investigation

Allen, C. E. prof. bot. Wisconsin.
Brooks, S. C. prof. zool. California.
Duggar, B. M. prof. phys. & econ. bot. Wisconsin.
Lewis, I. F. prof. biol. Virginia.
Robbins, W. J. prof. bot. Missouri.

Instruction

Drouet, F. res. fel. Yale.
Prescott, G. W. asst. prof. biol. Albion.
Taylor, W. R. prof. bot. Michigan. in charge.

INVESTIGATORS

Abramowitz, A. A. res. asst. Harvard. Br 122.
Algire, G. H. res. fel. anat. Maryland. Rock 6.
Amberson, W. R. prof. phys. Maryland Med. Br 109.
Anderson, Katherine res. tech. Vanderbilt Med. Br 122D.
Anderson, R. S. res. assoc. phys. Princeton. Br 225.
Angerer, C. A. instr. zool. Pennsylvania. Br 111.
Appel, F. W. assoc. prof. biol. St. John's (Md). Lib.
Arena, J. F. de la aux. prof. biol. Havana (Cuba). Br 330. D 302.
Armstrong, Louise S. res. asst. anat. Syracuse. Br 318. D 215A.
Armstrong, P. B. prof. anat. Syracuse. Br 318. D 215A.
Bailey, P. L., Jr. asst. prof. biol. City of N. Y. Br 9.
Baker, L. A. res. asst. Lilly Res. Labs. Br 319.
Ballard, W. W. asst. prof. zool. & anat. Dartmouth. OM 40. D 101A.
Ballentine, R. grad. Princeton. Br 231.
Barth, L. G. asst. prof. zool. Columbia. Br 228.
Bedichek, Sarah assoc. prof. biol. N. Texas Agr. Br 110. A 204.
Belcher, Jane C. grad. asst. zool. Missouri. Br 335. K 3.
Belda, W. H. grad. zool. Hopkins. OM Base. k.
Bender, J. C. res. asst. Swarthmore. Br 9. Dr 14.
Bernstein, F. prof. biom. New York. Br 305.
Bernstein, Marianne asst. Br 305. D 314.
Bertalanffy, L. von priv. docent. Vienna (Austria). L 21.
Bertalanffy, Maria M. von res. asst. Vienna (Austria). L 21.
Birnbaum, S. M. grad. Cincinnati. Br 341.
Birnbaum, W. Z. res. asst. biom. New York. Br 305.
Bishop, D. W. instr. zool. Pennsylvania. Br 219. Dr 10.
Bissonnette, T. H. prof. biol. Trinity. OM 28. D 108.
Black, D. C. res. assoc. zool. Swarthmore. OM 2.
Blum, H. asst. surg. New York Med. Br 328.
Boche, R. D. res. asst. emb. Carnegie Inst. (Baltimore). Br 339. (Aug. 1).
Bodian, D. Nat. Res. fel. med. Michigan. Br 217j.
Boettiger, E. G. grad. biol. Harvard. Br 110.

Botsford, E. Frances asst. prof. zool. Connecticut (New London). Br 110.
Bowen, W. J. fel. zool. Hopkins. Br 329.
Bozler, E. asst. prof. phys. Ohio State. Br 304. D 104.
Bradley, H. C. prof. phys. chem. Wisconsin. Br 122A.
Brambel, C. E. instr. zool. Hopkins. Br 301. (Aug. 14).
Brill, E. R. grad. biol. Harvard. 217l.
Bronfenbrenner, J. J. prof. bact. & immun. Washington Med. (St. Louis). Br 234.
Brownell, Katherine A. res. asst. Ohio State. Br 304. (Aug. 1).
Buck, J. B. res. asst. emb. Carnegie Inst. (Baltimore). Br 339. Dr 6.
Budington, R. A. prof. zool. Oberlin. Br 218.
Burnett, J. M. grad. bact. Washington Med. (St. Louis). Br 234.
Burton, A. C. fel. med. physics. Pennsylvania. L 29.
Cable, R. M. asst. prof. parasit. Purdue. Br 115. D 306.
Calkins, G. N. prof. proto. Columbia. Br 331.
Carothers, E. Eleanor res. assoc. zool. Iowa. Br 216. A 206.
Carpenter, R. L. prof. zool. Tufts. Br 106.
Castle, Ruth M. asst. zool. Vassar. OM 29. W E.
Cecil, S. asst. biochem. Vanderbilt Med. Br 122D.
Chambers, E. L. res. asst. biol. New York. Br 328.
Chambers, R. res. prof. biol. New York. Br 328.
Cheney, R. H. prof. biol. Long Island. Br 118. A 302.
Child, G. instr. biol. Amherst. Br 204.
Churney, L. instr. zool. Pennsylvania. Br 111.
Claff, C. L. res. assoc. biol. Brown. OM 38. A 208.
Clark, Eleanor L. res. asst. anat. Pennsylvania Med. Br 117.
Clark, E. R. prof. anat. Pennsylvania Med. Br 117.
Clowes, G. H. A. dir. Lilly Res. Labs. Br 328.
Cohen, A. res. fel. zool. Yale. Br 217e. D 111.
Cole, E. C. prof. biol. Williams. Br 311. Ka 1.
Cole, K. S. assoc. prof. phys. P. & S. (Columbia). Br 114. A 105.
Commoner, B. asst. biol. Harvard. Br 121.
Conklin, E. G. emer. prof. biol. Princeton. Br 321.
Cooper, K. W. fel. zool. Columbia. Br 225. K 9.
Cooper, Ruth S. asst. zool. Columbia. Br 225. K 9.
Copeland, D. E. asst. biol. Harvard. OM 41.
Copeland, M. prof. biol. Bowdoin. Br 334.
Cornman, I. fel. biol. New York. Br 332.
Costello, D. P. asst. prof. zool. North Carolina. Br 125. D 305.
Costello, Helen M. res. asst. zool. North Carolina. Br 125. D 305.
Cowles, R. P. prof. zool. Hopkins. Br 301. (Aug. 14).
Crawford, J. D. Milton Acad. (Mass.). Br 309.
Croasdale, Hannah T. tech. asst. zool. Dartmouth. Bot 2.
Crowell, P. S., Jr. instr. zool. Miami (Ohio). OM 25.
Curtis, H. J. assoc. phys. Columbia. Br 114. A 101.
Curtis, W. C. prof. zool. Missouri. Br. 342. (Aug. 1).
Denny, Martha instr. zool. Connecticut (New London). L 22.
Dienes, Priscilla res. asst. Dr. Bronfenbrenner. Br 234.
Donnellon, J. A. grad. zool. Pennsylvania. OM Base. f.
Dowding, Grace L. res. asst. Maryland Med. Rock 6.
Downs, J. H. Colgate. OM Base. d.
Drouet, F. res. fel. bot. Yale. Bot 23.
Dumm, Mary E. grad. biol. Swarthmore. Br 9. H 8.
Duryee, W. R. res. assoc. biol. New York. Br 312. D 215B.
Dziemian, A. J. grad. phys. Princeton. Br 231. Dr 1.
Eftman, H. asst. prof. zool. Columbia. Br 322. (Aug. 1).
Elwyn, A. assoc. prof. neur. P. & S. (Columbia). Br 343.
Failla, G. physicist. Memorial Hosp. Br 306.
Fennell, R. A. instr. zool. Michigan State. Br 217k. D 201A.
Ferguson, F. P. asst. biol. Wesleyan. Br 210. D 214.
Ferguson, J. K. W. asst. prof. phys. Ohio State. OM 3. K 5.
Fisher, K. C. asst. prof. exp. biol. Toronto. OM 4. Ka 1.
Florkin, M. prof. biochem. Liège (Belgium). Br 222. D 308.
Forbes, H. S. assoc. neuropath. Harvard Med. Br 313.
Foster, R. Milton Acad. (Mass.). Br 309.
Frank, J. A. Yale Med. 217f.
Frisch, J. prof. biol. Canisius (Buffalo, N. Y.). OM 45.
Fry, H. J. visit. invest. cyt. Cornell Med. OM Base.
Funkhouser, Elisabeth M. J. res. asst. biol. Swarthmore. Br 9.
Furth, J. asst. prof. path. Cornell Med. Br 340. (Aug. 1).
Garrey, W. E. prof. phys. Vanderbilt Med. Br 215.
Gettemanz, J. F. lab. asst. Rockefeller Inst. (Princeton). Br 206.
Gilman, L. C. instr. biol. Hopkins. Br 110. Ka 23.
Glaser, O. prof. biol. Amherst. Br 204.
Goldin, A. grad. zool. Columbia. Br 314. Ka 1.
Goodrich, H. B. prof. biol. Wesleyan. Br 210. D 107.
Gordon, H. res. assoc. New York. Br 312.
Grabar, P. chief lab. Institut Pasteur (Paris, France). Br 107. D 206.
Grand, C. G. res. assoc. biol. New York. Br 328.
Grant, R. lect. phys. McGill. Br 217h. D 318.
Grave, C. prof. zool. Washington (St. Louis). Br 327. Ka 2.
Gray, P. lect. emb. Edinburgh (Scotland). L 27. D 210.
Guthrie, Mary J. assoc. prof. zool. Missouri. Br 335.
Guttman, Rita grad. phys. P. & S. (Columbia). Br 114.
Hadley, C. E. assoc. prof. biol. Montclair State Teachers'. OM 29. D 204.
Hall, T. S. grad. zool. Yale. Br 323. (Aug. 15).
Hamburger, V. asst. prof. Washington (St. Louis). OM 39.
Hamdi, T. N. grad. phys. Pennsylvania. OM 3.
Hanson, F. B. assoc. dir. nat. sci. Rockefeller Foundation. D 312.
Harris, D. L. instr. zool. Pennsylvania. Br 111.
Hartman, F. A. prof. phys. Ohio State. Br 304. D 219.
Harvey, E. N. prof. phys. Princeton. Br 116.
Harvey, Ethel B. invest. zool. Princeton. Br 116.
Hatch, Cleora tech. Cornell Med. OM Base. W H.
Hauber, U. A. prof. biol. St. Ambrose (Davenport, Iowa). OM 45.
Heilbrunn, L. V. assoc. prof. zool. Pennsylvania. Br 220.
Henshaw, P. S. biophys. Memorial Hosp. Br 344. D 102.
Hiatt, E. P. res. fel. phys. Maryland Med. Br 109. D 201B.
Hickson, Anna K. res. chem. Lilly Res. Labs. Br 319.
Hiestand, W. A. assoc. prof. phys. Purdue. Br 115.
Hill, E. S. inst. biochem. Washington (St. Louis). L 31.
Hill, S. E. asst. gen. phys. Rockefeller Inst. Br 209.
Hinchey, M. Catherine grad. zool. Pennsylvania. Br 217d. A 307.
Hobson, L. B. grad. zool. Cincinnati. L. 26. Ka 2.
Hodge, C. asst. prof. zool. Temple. Bot. D 205.
Hodgkin, A. L. dem. phys. Cambridge (England). Br 114.

Hollingsworth, Josephine grad. zool. Pennsylvania. OM Base. i. W A.
Hopkins, D. L. res. asst. zool. Hopkins. Bot 5.
Horn, E. C. asst. biol. Trinity. OM 28. Ka 3.
Howell, C. D. prof. biol. Elizabethtown (Pa.). Br 329.
Hughes, Elizabeth D. lect. zool. Barnard. OM 22.
Hughes, R. D. asst. zool. Columbia. S. K 12.
Hunninen, A. V. prof. biol. Oklahoma City. Br 217o.
Hunter, G. W. asst. prof. biol. Wesleyan. Br 210. (Aug. 10).
Hunter, Laura N. asst. prof. zool. Pennsylvania Col. for Women. Br 217b. W F.
Hutchens, J. Lilly Res. Labs. Br 333.
Hutchings, Lois M. teach. biol. Weequahic H. S. (N. J.). OM Base. h.
Hutchins, L. W. grad. asst. zool. Yale. Br 323.
Irving, L. prof. biol. Swarthmore. OM 2. D 213.
Jacobs, M. H. prof. gen. phys. Pennsylvania. Br 205.
Jeffers, Katherine R. instr. zool. Duke. Br 335.
Jenkins, G. B. prof. anat. George Washington. OM 46.
Johlin, J. M. assoc. prof. biochem. Vanderbilt Med. Br 122D.
Jones, E. R., Jr. assoc. prof. zool. William and Mary. Br 311. D 310.
Jones, Ruth M. instr. bot. & zool. Swarthmore. Br 9.
Karady, S. asst. prof. phys. Francis Joseph (Hungary). Bot 3. A 104.
Keefe, E. L. res. asst. Washington (St. Louis). OM 39.
Keil, Elsa (Mrs. F. Sichel) asst. prof. zool. Rutgers (N. J. Col. for Women). OM 4. K 8.
Kemp, Emily J. instr. phys. Maryland Med. Br 109.
Kidder, G. W. asst. prof. biol. Brown. OM 21.
Kiese, M. asst. Pharmocological Inst. Berlin. Br 107. D 110.
Kille, F. R. asst. prof. zool. Swarthmore. OM 31. D 208.
Kindred, J. E. assoc. prof. hist. & emb. Viriginia. Br 106. (Aug. 1).
Knowlton, F. P. prof. phys. Syracuse Med. Br 226.
Kopac, M. J. res. assoc. New York. Br 328. A 106.
Korr, I. M. instr. phys. New York Med. Br 127.
Krahl, M. E. res. chem. Lilly Res. Labs. Br 333.
Krieg, W. instr. anat. New York Med. Br 315.
Kriete, B. C. grad. asst. zool. Cincinnati. L 26.
Kunitz, M. assoc. Rockefeller Inst. (Princeton). Br 206.
Lambert, Barbara grad. asst. phys. Mt. Holyoke. Br 122B. W C.
Lancefield, D. E. assoc. prof. biol. Queen's (Flushing, N. Y.). Br 322.
Levenson, A. S. grad. biol. Pittsburgh. Rock 7. Ka 21.
Levin, L. Cincinnati Med. Br 341. Dr 2.
Levy, M. asst. prof. chem. New York Med. Br 332.
Lewis, Lena res. asst. phys. Ohio State. Br 304. D 106.
Liebman, E. Fisheries Service of Gt. Brit. (Left).
Lillie, F. R. prof. emb. emer. Chicago. Br 101.
Lillie, R. S. prof. gen. phys. Chicago. Br 326.
Lipman, H. J. grad. asst. zool. Pittsburgh. Rock 7. Ka 21.
Loeb, L. prof. path. emer. Washington Med. (St. Louis). L 28.
Lucas, A. M. assoc. prof. zool. Iowa State. Br 223. K 1.
Lucas, Miriam S. Iowa State. Br 223.
Ludwig, F. W. grad. zool. Pennsylvania. OM Base. e. A 303. (Aug. 1).
Lyon, Rhea C. res. asst. Maryland Med. Rock 6.
McCann, L. P. grad. asst. bot. Maryland. Bot 1.
McClung, C. E. prof. zool. Pennsylvania. Br 219. A 201. (July 31).
McCurdy, H. G. res. asst. psych. Duke. Br 8.
McCurdy, Mary D. grad. zool. Duke. Br 8.
McDoneld, Margaret R. S. R. tech. Rockefeller Inst. Princeton). Br 206.
McFarland, Elsie L. instr. zool. Wheaton. Br 214.
MacLennan, R. F. assoc. prof. zool. Washington State. Br 218.
Magruder, S. R. asst. anat. Cornell Med. L 30.
Maloeuf, N. S. R. res. fel. phys. Yale. Br 323.
Martin, Mary S. Rochester Med. OM 2.
Martin, W. E. asst. prof. zool. Purdue, DePauw. Br 115.
Mast, S. O. prof. zool. Hopkins. Br 329.
Mathews, A. P. prof. biochem. Cincinnati. Br 341.
Matthews, S. A. asst. prof. biol. Williams. OM 27.
Mavor, J. W. prof. biol. Union. Br 315.
Mayo, Mercedes asst. prof. biol. Havana. Br 330.
Melland, Amicia M. res. worker Carnegie Inst. (Baltimore). Br 339. H 9.
Menkin, V. instr. path. Harvard Med. L 25.
Milford, J. J. grad. biol. New York. OM 41.
Miller, J. A. instr. anat. Michigan Med. Br 111. D 202.
Molter, J. A. instr. zool. Notre Dame. L 24.
Moore, Anna-Betty C. grad. zool. Columbia. Br 314.
Moore, J. A. asst. zool. Columbia. Br 314.
Morgan, Lilian V. Br 320.
Morgan, T. H. prof. biol. California Inst. Tech. Br 320.
Morrill, C. V. assoc. prof. anat. Cornell Med. Br 301.
Muller, H. J. Inst. Animal Genetics, Edinburgh. L 32.
Musser, Ruth E. Goucher. Br 336. H 3.
Naumann, R. V. fel. phys. New York Med. Lib.
Navez, A. E. prof. biol. Milton Acad. (Mass.). Br 309.
Netsky, M. res. asst. phys. Pennsylvania. Br 205.
Newton, W. H. reader phys. University Col. (London). Br 122C. A 203.
Nonidez, J. F. prof. anat. Cornell Med. Br 340.
Norris, C. H. grad. phys. Princeton. Br 127. Dr 4.
Northrop, J. H. mem. Rockefeller Inst. (Princeton). Br 206.
Obreshkove, V. prof. biol. Bard, Columbia. Br 123. D 217.
Olson, M. instr. zool. Minnesota. Br 111. K 7.
Orr, P. R. asst. prof. biol. Brooklyn. L 34.
Osborn, C. M. res. fel. zool. Harvard. Br 213.
Oster, R. H. asst. prof. Maryland Med. Br 336.
Osterhout, W. J. V. mem. Rockefeller Inst. Br 209.
Packard, C. asst. prof. zool. Columbia. Br 102.
Parker, G. H. prof. zool. emer. Harvard. Br 213. A 308.
Parmenter, C. L. assoc. prof. zool. Pennsylvania. Br 221. D 111.
Parpart, A. K. asst. prof. phys. Princeton. Br 231.
Patrick, Ruth assoc. curator microscopy. Acad. Nat. Sci., Philadelphia. Bot. D 205.
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McInnes, W. collector.

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Muse, R. collector.
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1938. Behavior of the epidermis in sponges (Microciona) when treated with narcotics or attacked by aquarium degeneration

J. Exp. Zool., v. 79, no. 2, Oct. 5

The epidermis including the marginal films, peripheral sheets of cytoplasm with a single layer of nuclei, is syncytial in reunition sponges (Microciona). The cytoplasm is, typically, reticulate in appearance.

Under the influence of chloral the syncytial epidermis may break up into cell-like pieces, owing to excessive vacuolar degeneration between nucleated regions. The degeneration is traceable to changes in the microscopic architecture of the cytoplasm. In such cases the nuclei seem to exert a directive influence on the progress of vacuolation. The cytoplasm eventually loses its optical structure and the nuclei also degenerate. Vacuolar degeneration may be diffuse without the production of cell-like pieces.

Chloretone has a similar but not identical effect. A non-nucleated area may divide into pieces, showing that nuclear control over vacuolation and consequent cytoplasmic division is unnecessary for such division. Aquarium degeneration may lead to results very similar to those induced by drugs.

Epidermal lines are again recorded and interpreted as structures produced through rearrangement of the meshes (alveoli probably) of the epidermal membrane.

Histology, epithelial tissue — Microciona

Physiology, degeneration

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(PHOTOGRAPH BY FRED S. HOWARD)

CONCERT OF THE WOODS HOLE CHORAL CLUB

Above: Members of the Choral Club after one of their numbers. **Below:** Ivan Gorokhoff, the conductor with his son Boris, librarian and daughter Galina, accompanist.

THE COLLECTING NET

Vol. XIII, No. 2 SATURDAY, JULY 23, 1938 Annual Subscription, $1.50 Single Copies, 30 Cents.

THE U. S. BUREAU OF FISHERIES LABORATORY AT WOODS HOLE

DR. PAUL S. GALTSOFF, *Acting Director*

This year, the fifty-fifth anniversary of the Fisheries Laboratory at Woods Hole, finds the activities of this Institution, so well known to the older generation of American biologists, greatly curtailed. Its facilities which a decade ago were available to a great number of scientists, now, with a few exceptions, are restricted to the needs of the Government. Although the present conditions have continued for the past six years, they are still considered temporary and no final decision has yet been made concerning the eventual fate of the oldest marine station of this country. Since, 1932, due to the limitation of the regular appropriations, no special allotment was set aside for the maintenance of the laboratory as such, and its formerly hospitable doors had to be closed to outside investigators. Thanks to the fact that the laboratory is closely connected with the marine hatchery which continues its operations, the fundamental equipment, as for instance, the salt water system, electricity, gas, aquaria, (*Continued on page 32*)

M. B. L. Calendar

TUESDAY, July 26, 8:00 P. M.

Seminar: Dr. B. H. Willier and Dr. Mary E. Rawles: Skin Transplants between embryos of different breeds of the fowl.

Dr. A. Cohen: Introduction by cauterization in the amphibian egg.

Dr. Victor Hamburger: The innervation of transplanted limbs of chick embryos.

Dr. Paul Weiss: The effect of mechanical stress on cartilage differential in vitro.

MONDAY, July 25, 1:30 P. M.

Discussion: Professor F. Bernstein: Application of statistical methods to biological problems.

FRIDAY, July 29, 8:00 P. M.

Lecture: Dr. Eduard Uhlenhuth: A quantitative approach to the secretion process of the thyriod.

THE SELECTIVE RELATION BETWEEN CENTERS AND PERIPHERY IN THE NERVOUS SYSTEM

DR. PAUL WEISS

Associate Professor of Zoology, University of Chicago

In order to produce a coordinated movement, e.g., of a limb, the central nervous system (CNS) must set into action a definite array of muscles in a definite temporal sequence and with a definite gradation of intensities. Comparing the muscles to the players of an orchestra, the CNS must conduct them according to a definite score. What provisions are there made to insure that each player will receive his cue correctly, as destined, and at the proper time? This question has rarely been asked, and an answer, if indeed articulate, has usually been given in terms of a supposed innate stereotypism of the central-peripheral connections; the conductor was assumed to know which player (muscle) lies at the end of which line of communication (peripheral nerve). Inadequacies of the peripheral performance—dissonances, as it were—were assumed to be corrected by the CNS

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THE MARINE BIOLOGICAL LABORATORIES OF WOODS HOLE

on the strength of back reports from the periphery by way of the sensory nerves. This view, although largely conjectural, has enjoyed general recognition for its plausibility.

But experiments conducted by the author since 1920 have increasingly emphasized an utterly different conception of the relation between centers and periphery. It was discovered that the CNS and each individual muscle remain in strictly selective communication even after the communication lines have been exchanged and confused at random. The method to ascertain this fact has been to "listen in" to the activity of the CNS by tapping some of its peripheral connections by one or several supernumerary muscles. We take, for instance, a limb muscle of an adult toad, e.g., a gastrocnemius muscle, and transplant it to an indifferent position, e.g., on the back, then provide it with a strange limb nerve, e.g., a nerve withdrawn from an antagonistic muscle, and allow the animal to recover. What will the function of the graft be? Will it react according to the former function of its nerve, or will it do what the other muscles do in the same location? Neither expectation is correct. The transplant behaves according to its *name*. The grafted gastrocnemius contracts always together with the normal gastrocnemius of the limb in whose nerve plexus it shares. A grafted semitendinosus muscle, under otherwise identical circumstances, contracts in unison with the normal semitendinosus, and similarly every supernumerary muscle contracts synchronously with precisely the *synonymous* muscle of the normal limb. Apparently the grafts somehow detect selective impulses given out from the centers for their own kind.

This principle of "homologous response" or "selective response" of muscles has been amplified by studies on transplanted supernumerary limbs. In a transplanted limb hooked to a random portion of the normal limb nerve plexus every muscle contracts at precisely the same time as the synonymous muscle in the normal near-by limb. Consequently, every movement of a left limb transplanted next to a left limb is an exact duplication of the simultaneous movement of the latter, while the simultaneous movements of a normal left limb and a right limb grafted next to it are exact mirror images of each other. If the normal left limb is simply replaced by a right limb, every muscle of the graft still reacts in the time order in which the synonymous muscle in the removed normal limb would have come due, which leads to very untoward consequences for the animal as whole. For, whereas the CNS discharges the "calls" for the individual muscles in the sequence devised for the anatomy of a normal left limb, the reversed arrangement of the muscles in the graft which is a right limb causes the movement to turn out in the reverse sense. If the animal intends to walk forward and discharges the impulse pattern provided for this act, the actual peripheral execution produces a movement backwards. That is, the peripheral effect is correct from the standpoint of the individual muscles, but it is wrong from the standpoint of what would serve the body. No adjustment or "re-education" has ever been observed.

When we say, a muscle responds according to its name, we mean, of course, according to some constitutional property which is individually characteristic and discriminative of that particular muscle and distinguishes it from all other muscles but the synonymous and homologous ones. This specific constitutional property may lie in a specific biochemical differential. By virtue of this property, *each muscle specifies its motor nerve*, turning it gradually into a selective receiver for central impulses which are of the form adequate for this kind of muscle. If a nerve is severed from its muscle and reconnected with a different muscle, it loses its old specificity and acquires the one characteristic of the new muscle. It is in this way that the CNS receives information as to what sort of muscle lies at the end of what line. Sensory influences have nothing to do with the establishment of the selective relation between center and periphery; for, the phenomena of homologous response in transplanted limbs are obtained even if sensory control is radically eliminated by the removal of the sensory roots and ganglia. The rate of the gradual re-specification of the motor neurones by their new muscles seems to depend on the age of the individuals; in older individuals (amphibia) it may require months. The nature of the process is unknown, but a resemblance between muscle-nerve specification and antigen-antibody correspondence is vaguely suggested.

The nature of the central "score" from which the specified motor neurones pick up their cues is likewise unknown. Whether it consists of a sequence of electrical states or chemical activities, one thing remains certain: namely, that it operates by means of as many different specific forms or modes of activity as there are individual muscles to be controlled within the district. Each half

The Collecting Net has been entered as second-class matter July 11, 1935, at the Post Office at Woods Hole, Mass., under the Act of March 3, 1879. It is devoted to the scientific work at marine biological laboratories. It is published weekly for eight weeks between July 1 and September 15 from Woods Hole, and is printed at The Darwin Press, New Bedford, Mass. Its editorial offices are situated on Main Street, Woods Hole, Mass. Between June 1 and October 1 communications should be addressed to Woods Hole, Mass.; at other times they should be directed to The Collecting Net, Garrison, N. Y. Single copies, 30c; subscription, $1.50.

of the spinal cord contains the "center" for its corresponding periphery; neither half can deal with the other's periphery except by using the discharge mechanism of the other side as an intermediary. Furthermore, under physiological conditions the actions of a given center remain confined to the local district in which they are generated; there is, for instance no spread of the central actions of the limb district into head or trunk segments.

The reported facts are based on extensive physiological studies of the function, and histological studies of the nerve supply, of the transplants. The most recent work has been published in volumes 66 and 67 of the *Journal of Comparative Neurology*, and a comprehensive review of the experiments and pertinent literature will be found in vol. 11, *1936*, of the *Biological Reviews* (Cambridge). To these publications the reader may be referred for further evidence and information on the subject in those points which have not been touched in this report.

THE U. S. BUREAU OF FISHERIES LABORATORY AT WOODS HOLE

(Continued from page 29)

etc., as well as buildings and grounds, have been kept in good condition and can again be used if funds for the wider operation of the laboratory become available. In the meantime a few improvements have been made and some antiquated equipment has been replaced. This year the outlook for the future appears brighter for it is expected that through a special relief project considerable improvements and repairs will be made in the laboratory buildings and grounds. The project plans submitted for the approval of the Budget Bureau include the installation of new salt water pipes, the enlargement and rebuilding of the aquarium, major repairs of the buildings and the construction of a new shallow draft motor boat suitable for work in local waters. At present the laboratory serves as a summer headquarters for the Shellfisheries Investigations of the Bureau and as a station from which certain phases of the North Atlantic Fisheries investigations are conducted.

The Shellfisheries Investigations during the present year consist of experimental studies of the physiology of reproduction and the metabolism of the oyster. The laboratory is well equipped for this purpose with specially built apparatus which automatically records the shell movements and the rate of flow of water through the gills of the mollusks placed in the metabolism chambers. The hatchery tanks on the first floor of the laboratory building, not used during the summer for hatching, are utilized by the investigators for keeping and rearing the experimental animals. A large number of these tanks are now being used for experiments in sex reversal of the adult oysters. The laboratory maintains several cultures of diatoms and green marine algae used as food for the larvae of mollusks and other marine invertebrates.

The public Aquarium continues to be a great attraction to the residents and visitors at Woods Hole. During last summer between fifty and sixty thousand people came to see the exhibits and during this past month the number of visitors who registered in the Aquarium book reached 2,398. It is estimated that about 2,500 people passed through the Aquarium doors over the 4th of July week-end. At least 1,205 of them left their names in the registration book. The proposed changes and improvements in the aquarium building will permit the enlargement of the exhibits and will greatly increase its educational value.

The library of the laboratory contains a number of publications not found on the stacks of the Marine Biological Laboratory. During the present summer the books are being put in order and upon the completion of the proposed repairs to the building, the library will again be open to the public.

Thus, it is hoped that in the near future the station will resume its full activity in marine research and will again carry on the work for which it was established by its famous founder, Professor Spencer Baird.

THE COLOR CHANGES IN ANIMALS AND NEUROHUMORAL TRANSMISSION

Report of a Lecture by Dr. George H. Parker
Emeritus Professor of Zoology, Harvard University

In the simplex reflex arc, so well known to the student of elementary biology, stimuli are received by sensory structures called receptors and are transformed into impulses which are carried to the central nervous system by way of sensory neurones. In the brain or spinal cord the impulse travels from the sensory neurone across the synapse to a motor neurone which in turn leads to the numerous effector mechanisms. Among these are muscles, glands, cilia, chromatophores,

nettle cells, luminous organs, electric organs, etc.

Interest in the synapse is twofold. It represents a certain loss of time in excess of that necessary for actual transmission of the impulse along a neurone. Here also is polarization; impulses are unidirectional, passing across in one direction and unable to return because of the synapse's valve-like action.

Theories of how impulses are transmitted across the synapse may be grouped under two headings: 1) electrical, in which the difference in electrical potential at opposite sides of the synapse causes an impulse to pass over from the sensory to the motor nerve and out to the effectors more or less directly, and, opposed to it, 2) the chemical, in which sensory end products act across the synapse on motor cells. Thus it is a matter of electrical impulses versus a substance produced on one side to excite the other. This substance likewise influences the peripheral apparatus, chromatophores which are familiar structures and will will be discussed this evening.

What light do these throw on nerve actions?

The activity of these chromatophores, organs of color change, was known to the ancients. Aristotle observed color changes in the chameleon and the devil fish but the subject was not studied fully until during the past century.

Color changes may be taken up in three main groups: the squids or devil fishes, which present remarkable color changes by their color sacs which are expanded and contracted by ringlets of smooth muscles; the crustaceans, with their variously colored cells in which the expansion and contraction are not muscular but the result of flowing protoplasm; and the lower vertebrates, fishes, frogs, toads, lizards. The higher vertebrates exhibit practically no color change. As an example of the lower vertebrates, the smooth dogfish presents a clear picture of chromatophore expansion and contraction. In the former, the fish looks dark and sleek; in the latter, it takes on a pale pinkish cast. In both, the melanophores present striking characteristics.

The situation in frogs interested the great scientist, Lister, in his early youth. In 1858, he experimented and found that frogs from which he had removed the eyes took on a dark tint and remained dark irrespective of background. This led him to believe color change depended on the eye and was purely a nervous reflex. Many other investigators agreed with him.

Pouchet, (1872-'76) working on fishes, narrowed this response to the action of the sympathetic or autonomic nervous system rather than the nervous system in general. Whenever he cut the sympathetics he found permanent darkening occurred.

In many fishes the changes are very marked. Dr. Mast working on flatfishes showed that they could adapt to various colors and even to patterned backgrounds, not actually copying the patterns but showing definite likenesses.

The end of the period of nervous interpretation came around 1920. Before that almost everyone accepted the nervous character of the phenomenon. Work of Adler, Smith, Allen, Atwell, etc. from 1914 on presented the beginning of a change to a hormonal theory. Hogben, studying the pituitary in frogs, found that the removal of this gland caused a paling condition. His work with Winton in 1922 showed that pituitary removal caused a permanently pale condition whether the background was light or dark. Injections of pituitary darkened the animals. He showed that color changes in frogs were controlled not by nerves but by means of this gland in the brain region. His experiments indicated that impulses travel from the eyes to the brain and in turn influence the pituitary to secrete a melanophore-dispersing substance carried by the blood to act on the peripheral apparatus. Hogben thus demonstrated the control of chromatophores by hormones. He concluded that color responses in fishes were controlled by nerves, in amphibians by hormones and in lizards by nerves.

Color change mechanisms may be illustrated by reference to three specific animals: frogs, smooth dogfish and catfish.

Color change in the frog is not activated by peripheral nerves. The eye by way of the brain influences the pituitary to give off its secretion of intermedin into the blood and to produce a darkening of the animal. A light condition is assumed in the absence of intermedin. This state of affairs was accepted by Hogben and confirmed by work done by Miss Scatterty and myself at the Harvard laboratories. The condition is strictly humoral. Many other animals, such as the lamprey eels, most shark and rays, react similarly according to work by Young, Miss Wykes and myself. Kleinholz, working in our laboratories, has found the same is true of the small Carolina lizard, Anolis, and there are scattered cases such as shrimps and prawns in which, lacking pituitaries, a substance is secreted by the eye stalks, which brings about color changes without the aid of nerves.

However there is not necessarily only one neurohumor. Hogben and Slome, in their work on Xenopus in 1931-36, postulated two substances. The B or black substance from the intermediate lobe of the pituitary-intermedin, and a W or white substance from the pars tuberalis. Dr. Soderwall has found two in frogs. I believe there is a fair chance that in his experimental work, he has excited the pituitary—specifically the intermediate lobe—by operations when he thought he was destroying the part which has only to do with whitening.

Crustaceans may have several substances some of which are secreted by the eye stalks. However, these may all be humoral. The color changing substances are carried in the blood, and nerves are not directly concerned.

Working with the smooth dogfish, Mustelus, Lundstrom & Bard in 1932 showed the presence of intermedin accounted for the dark phase but left open the question of how the light phase developed. Miss Porter and I showed in the same animal that cutting of nerves in the fin produced light bands and a local lightening, thus demonstrating that paling depended on nerves. Electrical stimulation produced the same result. Paling was the consequence of nerves; darkening, a humor reaction.

The catfish, however, presents a more complex picture though the color contrasts are striking. The pale condition may be due to two factors: 1) concentrating nerve fibers which contract the melanophores—electrical stimulation of the brain pales the fish as a consequence of nerve action; 2) furthermore it is possible by the action of an adrenalin-like substance which is released when the catfish is much excited and results in a sudden paling. This is called by students "excitement pallor."

Darkening, on the other hand, may be produced in the catfish in three distinct ways: 1) by the action of intermedin; 2) by dispersing nerve fibers, and 3) by no stimulation whatever but by a condition which may be termed a "resting state." There are then in all five possible means of change, two nervous, two humoral and one, inherent. In the common killifish, Fundulus, three of these five factors are presented. It pales because of concentrating nerves and darkens through the influence of dispersing nerves and by a slight amount of intermedin.

That color change in fishes involves a double innervation, two kinds of nerves, one for the pale and one for the dark state, is established by no direct evidence other than simple histology. The copious aborization of nerve terminals surrounding chromatophores, pictured by Ballowitz, shows sufficient innervation to justify a theory of double innervation and also represent the source of neurohumor production. These endings are very unlike nerve endings in muscle where fewer terminals are intimately attached to the fibers. It is more than likely that such a complex system exists also in the horn toad and the chameleons. Activation then in fishes is mixed, partly nervous and partly hormonal.

Some idea of the way nerves act may be presented in the blanching of dark denervated bands in Fundulus. This fish has distinct light and dark phases, is naturally rather pearly with great transparency. If nerves are severed across the tail find of a pale fish, a dark caudal band is produced by the expanded melanophores. In the edges of this new band one finds melanophores remarkably expanded in contrast to the contracted cells in adjacent innervated areas. The line of demarcation is very sharp. Such a band may fade so as to disappear completely. This process progresses with great regularity, the edges paling first leaving the dark band narrower until the central dark axis finally disappears. If a band one millimeter wide is cut, it disappears in about a day, a band two millimeters wide remains two days. These may be seen only in pale fish. In a dark fish, the dark band is of course indistinguishable from the rest of the body shade.

There is then an invasion of an activating pale substance into the dark band, a lateral invasion from the outside. This was tested by operations in which I cut the tail to produce newly denervated bands flanking either side of a previously faded denervated band. These resulted in general darkening of the originally faded band midway between the two new bands. This experiment convinced me that something was coming in sidewise into the denervated portion of the fin. This substance is not water soluble. Blood has nothing to do with it.

Suspecting it would dissolve in fatty lipoid material in the cells, working on the natural oils, I believed I should be able to extract the substance by oil or ether from one type of fish and apply it under the skin of another. The skin of a light dogfish was taken, dried, extracted in ether and ground with oils and injected into a dark dogfish. A light area was formed in a day at the point of injection. Administration of intermedin redarkened the area but finally wore off and the area lightened again. To test that this reaction was due to an active substance of the skin, plain oil and water were injected on the other side of the fish. No light spot occurred, thus checking the fact that the active material was that extracted from the light dogfish.

Extract from a dark catfish injected into a light catfish produced dark areas with melanophores expanded. Thus nerves produce two substances, one light and the other dark, in one case in a dogfish in the other, a catfish. The extracts were crude but the effective material resists 110 degrees C., boiling in weak acid or alkali, is soluble in alcohol and oil, and is stable when dry for a year or more as that of the dogfish was kept a year at the Harvard Laboratories before it was brought back for use at Woods Hole. It is apparently something like a sterole.

In another experiment dogfish extract which paled another dogfish darkened catfish and frogs, presenting a curious contrast which is paralleled in crustaceans. Eyestalk secretions of the shrimp injected into shrimp caused paling but the same substance injected into a fiddler crab changed it

from light to dark. It is possible that extracts may contain several neurohumors but it is more probable that chromatophores of the fiddler crab are different in response from those of the shrimp.

Other neurohumors besides intermedin, adrenalin, oil soluble neurohumor, eye stalk substances are acetylcholin and sympathin.

Neurohumors may be defined then as hormones derived from the nervous system or appended glands activating other parts of the nervous system or its effectors. Water soluble neurohumors such as intermedin, adrenalin, etc. may be classed as hydrohumors; lipoid solubles such as the nerve terminal secretion, lipohumors.

Neurohumors offer a reasonable explanation of synaptic delay and synaptic polarization. A secretion would take more time than an electrical impulse to pass over the synaptic bridge. As secretions they naturally flow in only one direction, away from the secreting nerve ending, toward the non-secreting side of the synapse.

Neurohumors are certainly of several and perhaps numerous kinds. They occur at many neural junctions. Whether they occur at all such junctions is for future investigators to determine.

(This article is based upon shorthand notes of a lecture given by Dr. G. H. Parker on June 15, at the Marine Biological Laboratory.)

SOME EFFECTS OF TEMPERATURE ON THE FREQUENCY OF DIVISION IN "CHILOMONAS PARAMECIUM"

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Chilomonas paramecium is an excellent laboratory organism for experiments dealing with temperature. It thrives under sterile conditions on a medium made up of salts which are inorganic with the exception of sodium acetate. Its viable temperature range is wide, namely, from about 9° to 32° C., and can be transferred from one extreme in this range to the other and still survive quite well.

The isolation culture method was used in all the experiments. That is, one organism was isolated by transferring to a depression slide containing suitable nutrient media and the slide was put into the proper temperature. At the end of about 24 hours, the number of organisms present was ascertained. In these experiments, the usual procedure was to expose the chilomonads to 14° or 9.5° C. for a given time, and then transfer them by the isolation culture method to the other temperatures where daily observations were made on the frequency of division.

The first graph gave the values for the frequency of division at various temperatures after the organisms had become fully acclimatized to these temperatures, and showed that the frequency of division is zero at 9.6° C., that, as the temperature rises, the frequency of division increases until it reaches a maximum at 26°. The value of the frequency of division remains at this maximum value as the temperature rises further to 30.5° C., then decreases rapidly until it becomes zero at 35° C. Thus the optimum temperature range for reproduction is between 26° and 30.5° C., and at temperatures above and below this range, the frequency of division decreases. It is interesting to note that above 30.5° C. death is the cause of the decreased frequency of division, while below 26.° C., the decrease is actually due to the decrease in the frequency of division, because it is possible to keep the chilomonads at, for instance, 9.5° C. for five weeks, and during this time they live quite well although there is no reproduction at all.

The second graph showed how the lethal effect found at 30° and 35° C. is changed by prolonged exposures to 14° and 9.5° C. previous to the observations. The graph showed that as the exposures to these two low viable temperatures are prolonged, the total length of the life of the clone decreases and at the same time there is a decrease in the rate of reproduction or/and an increase in the death rate when the clones are subjected to 35° C. When the chilomonads were exposed to 14° C. for 1 week, they lived and reproduced at a decreasing rate when transferred to 35° C. for a total of nearly 168 hours, or nearly a week, but when they had been subjected to 14° C. for 1 week followed by 9.5° C. for three and four weeks respectively, and were then transferred to 35° C., they died within the first 48 hours with no reproduction whatsoever. The same phenomenon occurred at 30° C. but to a less striking extent. This experiment emphasizes the fact that the "lethal temperature" is influenced by the treatment the organisms have had previous to the observations, in addition, of course, to the two other factors that are well known, namely, the temperature itself, and the length of the exposure to the lethal temperature.

The third graph showed the effect of prolonged exposure in temperatures of 9.5° C. and 14° C. on the main frequency of division at various temperatures. At all temperatures tested, 30°, 27°, 23°, 18°, and 14° C., there was a slight decrease in the frequency of division as the exposure to 9.5° C. was prolonged previous to the observations. The mean, computed by averaging the frequencies in all temperatures after exposure to a given

treatment, shows that this decrease namely from 0.075 to .055 in the frequency of division is actually present and is statistically significant. It is likely that this decrease in the frequency of division has its source in the divisions during the first week of the observational period, because after a period at any viable temperature, the frequency of division assumes a constant value that is reproducible at that particular temperature.

There is an immediate effect on the frequency of division upon transfer of the organisms from 14° C. and 9.5° C. to various other temperatures. During the first 48 hours or so there is a gradual increase from no division at all (at 9.5° C.) up to a value that is equal to the mean frequency of division. This gradual increase indicates that the period of adjustment or period of change necessary to allow the chilomonads to live in a changed condition of temperature takes from 24 to 48 hours, depending upon the extent of the temperature change.

To sum up, I have attempted to point out four general observations:

First, the optimum temperature for reproduction in *Chilomonas paramecium* is between 26° and 30.5° C., and above and below this range, the frequency of division decreases until it becomes zero at 35° and 9.5° C., respectively.

Second, the lethal temperature is dependent to a great extent upon the treatment that the chilomonads have had previous to the observations at the lethal temperatures.

Third, as the chilomonads are exposed to low viable temperatures for prolonged periods of time, the frequency of division when transferred to other higher temperatures is slightly lower than is normal for the higher temperatures.

Fourth, there is a pronounced lag in the rate of change in the frequency of division when the organisms are transferred from a low temperature to a higher temperature.

(This article is based upon a seminar given at the Marine Biological Laboratory on July 12.)

ADJUSTMENT OF THE MARINE AMOEBA, "FLABELLULA MIRA" SCHAEFFER TO CHANGES IN THE TOTAL SALT CONCENTRATION OF THE OUTSIDE MEDIUM

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This amoeba is remarkable in its ability to withstand changes in the concentration of the medium. It lives equally well in sea water and in sea water diluted 20 times with distilled water. It has been cultured for a number of years in artificial sea water and for eight months in sea water diluted 20 times. It can be cultured and will grow in sea water diluted 50 times with distilled water, but for only two or three weeks and then it dies out. On the other hand in sea water cultures which have evaporated to such an extent that NaCl has begun to precipitate from solutions, they are frequently found to remain still active and to continue to feed.

I have described the vacuole system of this amoeba in Biodynamica Vol. 1, No. 34. The vacuoles are all potentially food vacuoles, but when extruded a large amount of fluid is eliminated at the same time as the fecal material. Thus the vacuoles seem to serve the same function as the contractile vacuole in the fresh water protozoa. Regardless of the dilution of sea water regular contractile vacuoles filled with fluid never appear.

The rate of elimination of fluid material, by vacuolar extrusion, by normal active feeding individuals which have been cultured in various dilutions of sea water up to 50 dilutions is inversely proportional to the concentration of the medium and directly proportional to the volume of the amoebae. If these amoebae are transferred from sea water to diluted sea water they increase in volume rapidly and subsequently decrease in volume until the volume is essentially the same in the diluted as it was in the original concentrated sea water. The reverse takes place if the amoebae are transferred from dilute to concentrated sea water. The volume decreases and subsequently increases to a volume in concentrated essentially the same as it was in the original dilute sea water.

When the amoebae have swelled due to dilution of the medium they shrink rapidly. The volume loss during this period of shrinkage is not nearly accounted for by the fluid eliminated from the cell by extrusion of vacuoles.

Once the amoebae have become adjusted to a given concentration of the medium they always decrease in volume when the concentration is increased slightly. Consequently when adjusted to a medium the cytoplasm has an osmotic value only slightly higher than that of the medium.

Summary. When the concentration of the medium is changed this amoeba adjusts itself to change by the loss or gain of osmotically active substances until the internal osmotic value is only slightly higher than that outside. Further the loss or gain of volume on changes of concentration is not accounted for by the action of the vacuoles. The functions of the vacuoles do not seem to include that of regulation of the water content of the balance of the protoplasm since this is regulated without their action. Yet the rate of elimination of fluid by the vacuoles of active, adjusted amoebae is inversely proportional to the concentration of the medium.

(This article is based upon a seminar report given at the Marine Biological Laboratory on July 12).

RHYTHMICAL CHANGES IN BLOOD FLOW THROUGH MUSCLES

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Rhythmical changes in the blood pressure of anesthetized animals have been described since they were first observed by Traube and by Hering. It has been thought that these waves represented a discharge from the vasometer center which was activated by influences which were transferred from the respiratory center. The blood pressure is, however, a complex resultant of cardiac activity and of the resistance offered by the peripheral blood vessels, and study of the blood flow through individual tissues is more likely to indicate the operation of the peripheral vascular bed and to suggest the way in which it is controlled.

I have examined blood flow in muscles and in other tissues by means of a heated electrical resistance wire type of flow meter which operates on the principle of the hot wire anemometer. In about 25% of the cats under chlorlose-urethane anesthesia rhythmical changes in arterial blood pressure were observed amounting to 10 or 20 mm. in height and in a period of from 4 to 2 per minute.

In muscles as the blood pressure rises the flow diminishes and the flow record indicated that the rhythmical changes in pressure originated in changes in the blood vessels of the muscles. Severing the voluntary nerves or the corresponding sympathetic supply changed the muscle flow waves in such a way that flow now passively followed pressure changes. In the brain flow likewise rhythmically and passively followed the pressure changes.

The rhythmical changes in pressure and flow were not closely related to the control of breathing; for if the breathing were altered by (1) the inhalation of CO_2 or a deficient O_2 mixture, or (2) by apnea caused by inflation of the lungs, or (3) by vagotomy the flow rhythm was not significantly changed.

The rhythmical change in flow was not closely related to the arterial pressure for it persisted practically unchanged (1) when the blood pressure was depressed by the Valsalva effect or (2) when blood pressure was raised by carotid sinus stimulation from clamping the carotids. The important respiratory afferents in the vagus, depressor and carotid sinus nerves were not essential, for the rhythm appeared after their denervation.

These observations indicate that the rhythmical changes in pressure originate largely in the vascular bed of the muscles and that they are activated over the sympathetic pathway. The rhythm must originate in a vasomotor center which is relatively independent of the control of respiration. The nature of the rhythmical activity in the periphery indicates the type of rhythmical activity within the center and suggests how the activity of a center may be examined by the behavior of the peripheral system which it controls.

(This article is based upon a seminar given at the Marine Biological Laboratory on July 19).

THE STEADY STATE FREQUENCY OF THE EMBRYONIC FISH HEART AT DIFFERENT CYANIDE CONCENTRATIONS

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A priori the frequency at which periods of activity occur in such an excitable tissue as the heart, might depend either on the rate at which recovery from the immediately preceding period of activity took place, or in the rate at which adequate stimuli were presented to the recovered tissue, or perhaps on both of these factors. In actuality however, from the evidence available it seems as though the heart rate is always determined by the rate at which adequate stimuli are presented to it, the frequency determining processes being normally resident in the sinus tissue. The observations to be described support this view and indicate several characteristics of the processes which are involved.

When intact embryos of salmon, trout or Fundulus are exposed to neutral solutions of cyanide, the frequency of the heart is observed to fall gradually with time, a new steady state being reached during which the frequency tends to be constant or to fall only slowly. The level at which the frequency is maintained during this period depends upon the cyanide concentration, and is lower the higher the concentration. The inhibition may be removed by returning the organisms to fresh water. The effect is usually maximal with N/1000 cyanide (up to N/250 was used). It amounts to a lowering of the frequency of the heart, in Fundulus, to approximately 27 per cent. of the normal. As the cyanide concentration is made more dilute than N/1000 the inhibition becomes less until at N/30000 it may be only just perceptible.

If at any given cyanide concentration, the portion of the frequency which has been inhibited is designated the "inhibited frequency," while the

extra portion of the frequency which would be inhibited if the rate reduction were maximal is designated the "uninhibited frequency," then the effect of different cyanide concentrations is described by the equation

$$\frac{\text{inhibited frequency}}{\text{uninhibited frequency}} = K\,[CN]^{-1.6},$$

K being a constant which varies with the temperature (Q_{10} = approximately 3) and [CN] the cyanide concentration. The accuracy with which this relation represents the data is measured by the difference between an observed value and the equivalent calculated one. This difference on the average is 3 per cent. of the theoretical value. The formula is an expression of the law of mass action which would apply to the case if cyanide were entering, and thus slowing, an essential step in a chain of reactions which contribute to the determination of the frequency. Such a mechanism as this is the one recognized to be operative in the inhibition of the oxygen consumption of tissues by cyanide, the essential step being the Warburg-Keilin respiratory system. The specificity of cyanide as a respiratory inhibitor makes it necessary to suppose that its effect on the heart beat frequency occurs by reason of its intereference with the normal pathway of the oxygen supply to some cellular process.

Our observation that the maximum inhibition produced by cyanide in trout and salmon embryos is only 45-50 per cent. compared with 73 per cent. in Fundulus, is most simply taken to indicate that the series of reactions which include the atmungsferment, is responsible for only 45-50 per cent. of the normal frequency in trout and salmon but for 73 per cent. of the normal in Fundulus.

It has been noted further that in salmon and Fundulus the maximum cyanide inhibition increases as the temperature rises which, if the interpretation is to be kept consistent, indicates that the velocity of the chain involving the cyanide sensitive system must increase faster as the temperature rises than does the velocity of the cyanide insensitive chain. Apparently the proportion of the oxygen consumption, or, as in the present case, the proportion of energy liberation as measured by the frequency of the appearance of stimuli to the heart muscle, is not a fixed quantity but varies with the conditions to which the cells are exposed. The loss of the cyanide sensitivity of the respiration of yeast as the cells become unsaturated, which several authors have reported, is undoubtedly an analagous situation.

In the experiments which we have reported here the heart beat frequency is observed to vary in much the manner which would be expected of the oxygen consumption of typical cells under similar circumstances. It can only be concluded that the effects which we observed on the frequency of the heart, were actually produced by virtue of the derangement by cyanide of the normal route taken by oxygen through the cells concerned with the establishment of the frequency. We have shown that the effect of cyanide is adequately described by a simple application of the law of mass action presumably because cyanide combines specifically and reversibly with an essential constituent, the Warburg-Keilin oxidase, of the oxidation-reduction systems in the cell. These findings, we believe, are in accord with the notion that in the pacemaking cells there is a constant building up of "excitement" (Eccles and Hoff) by at least two parallel series of reactions one of which includes the atmungsferment; and that the sudden dissipation of this "excitement" constitutes the stimulus which gives rise to each beat.

(This article is based upon a seminar presented at the Marine Biological Laboratory on July 19).

STUDIES OF THE REFRACTORY STATE RESULTING FROM REPEATED INJECTIONS OF ADRENAL EXTRACT

Miss Lena A. Lewis, Dr. Frank A. Hartman and Mr. Kenneth C. McConnell
Department of Physiology, Ohio State University

It has been reported previously from this laboratory that the injection of large amounts of adrenal extract into the normal human being and the normal dog causes marked changes in the electrolyte excretion. The effect is differential, the excretion of sodium and chloride being decreased and that of potassium and ammonia increased. The total base excreted remains essentially unchanged. It was further shown that repeated intravenous injections of adrenal extract eventually produce a condition in which there is no response. Harrop and Thorn, however, failed to observe the development of a refractory state following subcutaneous injections.

The conditions which lead to the development of a refractory state to adrenal extracts have been further investigated during the past year.

Normal adult male and female dogs, adult female cats and a young immature female pig were studied. All of the animals were on a constant diet and regime throughout the experimental period. The adrenal extracts used were Upjohn

Company extract, Wilson Company extract and extracts prepared in this laboratory according to the method of Cartland and Kuizenga.

Subcutaneous injections failed to develop a refractory state, even after many injections over a long period of time. (3 dogs used). These findings confirmed the observations of Harrop and Thorn.

Experiments in which a mixture of adrenal extract plus pig serum was injected subcutaneously repeatedly, gave no indication that the refractory state was due to haptene formation.

The refractory state was produced in one dog and one cat by repeated intraperitoneal injections.

Dogs which were refractory to beef adrenal extract, when injected with pig adrenal extract gave a typical electrolyte response. Similarly dogs refractory to both beef and pig adrenal extracts gave a response to horse or sheep adrenal extract. (4 dogs). The refractoriness developed to adrenal extracts is thus species specific.

A young pig which was injected repeatedly intravenously with pig and later with beef adrenal extracts failed to develop a refractory state. Her age may have been a factor in the failure to develop the refractory state as all other animals used were adults. Similar responses were produced by both species of extract. Sixty-four C.U. of pig adrenal extract produced a 75 per cent. retention of sodium on the initial intravenous injection, while a similar injection of beef adrenal extract produced an 81 per cent. decrease.

Due to the ease with which the refractory state is developed by intravenous and intraperitoneal injections it would appear unwise to use these modes for injection of adrenal extracts in the treatment of Addison's disease.

(This article is based upon a seminar report given at the Marine Biological Laboratory on July 19.)

ACTION POTENTIALS OF VISCERAL SMOOTH MUSCLES

DR. EMIL BOZLER
Assistant Professor of Physiology, Ohio State University

It has been shown previously that strips of visceral muscles respond to electric stimuli like single giant muscle fibers. The conduction of impulses in smooth muscle does not involve nervous structures and can only be explained by assuming syncytial connections between the smooth muscle cells. In agreement with this result, the action potentials of strips of intestinal and uterine muscle accompanying a propagated response consist of waves of negativity differing from nervous impulses only by the time factors concerned.

The spontaneous contractions of the uterus and the gut are tetanic contractions as shown by the observation that a burst of impulses accompanies each contraction. The maximum frequency of impulses found in the small intestine is eight per second, in the uterus four per second. A regular discharge of rather low frequency occurs during tonic contractions.

The peristaltic waves of the ureter of the guinea-pig are accompanied by bursts of impulses discharged at a surprisingly high frequency (about forty per second at 38°). However, the monophasic potential of the ureter of other species is a single slow negative variation. In the rat it lasts for 1.5 to 3.5 seconds and has a remarkable similarity with the monophasic potential of cardiac muscle. Diphasic potentials of the ureter of this species have typical "R" and "T" waves. The comparative study of the action potentials and of other related properties of the ureter lead to the conclusion that the slow potential variations are the equivalent of a burst of impulses which have fused into a continuous excitatory state.

(This article is based upon a seminar report given at the Marine Biological Laboratory on July 19.)

THE INDIANA UNIVERSITY BIOLOGICAL STATION

The program of the Indiana University Biological Station has been somewhat curtailed this summer due to the recent death of the Station Director, Professor Will Scott. The program of work which Dr. Scott had begun with the Indiana State Department of Conservation is being continued. Four graduate students, Mr. Albert Weyer, Mr. David Opdyke, Mr. Meredith Runner, and Mr. Richard Buckingham, are carrying on problems on the study of fish parasites, insect emergence, growth of fishes, and scale studies. This is part of a general program of biological survey which was being instituted by Mr. A. E. Andrews of the Division of Fish and Game and by the late Dr. Scott. The University feels fortunate in being able to continue this work so that no lapse in the research program will result before the appointment of Dr. Scott's successor. Professors T. W. Torrey and W. R. Breneman of the Indiana University Department of Zoology are aiding in the supervision of the work during this summer.

—W. R. Breneman

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris Gorokhoff, Bradford Chambers, and Garnette McClure.

Entered as second-class matter, July 11, 1935, at the U. S. Post Office at Woods Hole, Massachusetts, under the Act of March 3, 1879.

Introducing

Dr. Ludwig von Bertalanffy, privatdozent at the II. Zoologisches Institut der Universitat Wien (Vienna); Rockefeller Foundation Fellow at the University of Chicago.

Descended from a Hungarian noble family, Dr. von Bertalanffy was born in a town located near Vienna, Austria. He was educated at the universities of Innsbruck and Vienna, receiving his doctor's degree from the latter institution. He then became privatdozent at the University of Vienna, a position which he still holds, lecturing there on general biology and physiology. His readership in theoretical biology is the only one on the continent devoted explicitly to general problems and laws of biological phenomena.

Dr. von Bertalanffy, accompanied by his wife, Maria von Bertalanffy, arrived in America last October to work under a Rockefeller Foundation Fellowship at the University of Chicago. During the spring he visited a number of universities in this country in order to give lectures and to visit men working in his field. He came to Woods Hole in June, and is now at the Marine Biological Laboratory. He will remain there until September, returning to Europe probably a month later.

In addition to numerous papers, Dr. von Bertalanffy has written several books, one of which, "Modern Theories of Development," has been published in three languages. His "Theoretische Biologie," the first volume of which was published in 1932, gives a "synthetic representation of the theoretical results in modern biology." Other books include "Kritische Theorie der Formbildung" and "Das Gefüge des Lebens." He is editor of a series of monographs entitled "Abhandlungen zur exakten Biologie," which is being published at Berlin.

Dr. von Bertalanffy came to the United States:

(1) to study and correlate American developments in quantitative biology, a subject in which he is especially interested, having been engaged in developing an "organismic" point of view which he hopes will contribute to the reconciliation of mechanism and vitalism; (2) to continue experiments on basic processes connected with the growth of planarians—such as starvation, temperature, metabolism, and magnitude of interior surfaces—to further develop and verify his mathematico-physiological theory.

THE TREATMENT OF THE "WOOD" TICK BITE

Although no reason exists for undue alarm over the possibility of contracting Rocky Mountain Spotted Fever or tularemia from the bite of a tick, unusually prevalent this year in certain regions including the Cape and adjacent islands, it seems worthwhile to point out again the simple measures which can be taken by all—and which perhaps should be followed by those who find that they are unwittingly collecting ticks on their clothing, and later their bodies.

Prevention. If it is necessary to spend much time in the underbrush or in deep grass, high-topped boots and cotton outer garments soaked in kerosene should be worn.

Treatment. Most of us may collect only a few ticks as a result of a short walk "off the concrete" or during a beach party. The favorite feeding place is the back of the neck or the head. If exposed for the entire day these regions should be examined on at least two occasions (ticks ordinarily do not settle down immediately to bite); and the clothing and body should be thoroughly inspected at night, or sooner if convenient.

If a tick is found attached and sucking blood do *not*, as recently advised in a medical journal, pull it off at once. This may result in leaving the barbed chitinous proboscis in the wound, and the pressure of the fingers or forceps may squeeze out of the tick the infective coxal fluid or the (apparently) similarly infective intestinal contents. Rather attempt to persuade the tick to withdraw its proboscis and drop off by putting a drop of kerosene, creoline, salad oil, chloroform, or ether on its head; or a smear of lard or vaseline on the entire animal. The latter treatment stops up the breathing spiracles on the under edges of the middle of the body. By either treatment, the tick should drop off in a minute or two; but if necessary to pull, do so slowly and gently.

After removal, disinfect the bite by drilling gently in the hole a blunt toothpick or a rounded wooden matchstick which has been dipped into iodine solution, and follow up by applying iodine to the area surrounding the bite.

Clothing can be "deticked" by placing it for a few hours in a tightly closed vessel, in the bottom of which is a shallow pan containing several ounces of carbon tetrachloride or carbon disulfide.

—*Correspondent*

ITEMS OF INTEREST

Professor Edwin G. Conklin will give an informal talk on "The Biological Basis of Democracy" at the Penzance Forum on Sunday afternoon. Dr. Warbasse, who for many years has sponsored these gatherings, extends a cordial invitation to everyone at the laboratory.

The exhibit of the Spencer Lens Company on Water Street has been succeeded by the Bausch and Lomb Optical Company who will display their apparatus until July 29. Blakiston and Saunders are exhibiting their books in the lobby of the Brick Building of the M. B. L.

Dr. Philip B. Armstrong, last year professor and head of the Department of Anatomy of the University of Alabama Medical School has been appointed professor of anatomy at the School of Medicine of Syracuse University.

Dr. R. L. Carpenter, Assistant Professor of Anatomy at the College of Physicians and Surgeons of Columbia, has been appointed professor of zoology at Tufts College. He succeeds Prof. Herbert B. Neal, who is retiring from teaching after twenty-five years at Tufts.

Prof. Horace W. Stunkard, Professor of Biology at the University College of New York University, has been granted a leave of absence for the coming academic year. He left on the 6th of July for Hamburg where he will carry on research at the Institute for Ship and Tropical Diseases on the life history of a parasite, the anoplocephalid tapeworm. Prof. Stunkard's family is accompanying him on his trip to Hamburg.

Dr. and Mrs. G. H. A. Clowes and their two sons, George and Allan, left today for Europe aboard the liner *Rex*. Dr. Clowes, who is director of the research laboratories of the Eli Lilly Company, will attend the International Congress of Physiology, in Zurich. The rest of his family will travel through Italy, Germany, Switzerland, and England before they return on September 8.

ALLEGANY SCHOOL OF NATURAL HISTORY

Dr. Oscar W. Richards, Research Biologist at the Spencer Lens Company and formerly in charge of the Chemical Room at the Marine Biological Laboratory, is a member of the faculty of the Allegany School of Natural History, giving a series of lectures on Technique of the Microscope and conducting demonstrations for a class in this subject. Another innovation—among the courses offered is one in field ethnology of Eastern North American Indian Tribes. Dr. William N. Fenton who conducts this course is Assistant Professor in Social Studies at St. Lawrence University.

—Robert B. Gordon, Director

Dr. B. R. Coonfield, of Brooklyn College, Percival Bailey, Jr., of the College of the City of New York, and Morris Jones of Swarthmore are working this summer at the Tortugas Laboratory.

Dr. John H. Welch, instructor in zoology at Harvard University, is working at Bermuda during the month of July. He spent last summer at the Plymouth Laboratory in England and the winter working at Cambridge University.

Dr. and Mrs. Harry M. Miller, Jr. are visiting Woods Hole for the first time since 1924. Dr. Miller is assistant director for natural sciences in the Paris office of the Rockefeller Foundation and is in charge of the fellowship program for Europe. When he worked at the Laboratory last he was assistant professor of zoology at Washington University.

Dr. Daniel Ludwig, who will be working at the Laboratory in August, has been promoted from assistant to associate professor of biology at University College, New York University.

MOTION PICTURES IN THE AUDITORIUM

On Wednesday "biological" moving pictures were presented by investigators in the Auditorium both in the afternoon and evening. Dr. Paul Weiss showed his film "The selective response of transplanted muscles and limbs" after dinner. It covered the same general field as his article in the present issue of The Collecting Net.

Professor Robert Chambers and Dr. Duryee presented their pictures on "Micromanipulation studies on cells and nuclei" in the evening. They were shown especially for the classes in embryology and physiology although many persons not connected with the courses attended the exhibition.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
July 24	2:04	2:19
July 25	2:57	3:15
July 26	3:58	4:06
July 27	4:49	5:05
July 28	5:41	5:54
July 29	6:26	6:50
July 30	7:20	7:42

In each case the current changes approximately six hours later and runs from the Sound to the Bay.

THE WORK OF THE "ATLANTIS"

Columbus O'D. Iselin
Assistant Professor of Oceanography, Harvard University

The *Atlantis* returned on Tuesday from a cruise which lasted nearly seven weeks. The main object of the cruise was to secure additional routine observations for the cooperative investigation now being carried on with the Bermuda Biological Station. This study of the possible long period changes in strength of the major currents of the North Atlantic originated more than a year ago at the suggestion of a committee of the Royal Society. This committee has provided the Bermuda Laboratory with a small research vessel, the *Culver*, and also has sent out two British investigators. A five-year program has been worked out. However, during the first year and until the *Culver* could be made ready, it was agreed that the *Atlantis* would secure all the necessary periodic observations.

The scientific party, which left here on June 29th, consisted of the following: E. F. Thompson, the hydrologist for the Royal Society and Alfred Woodcock, Dean Bumpus and Drayton Carritt, technicians from the Woods Hole Oceanographic Institution. During the first ten days the subsurface distribution of temperature and salinity was observed in detail along a section extending from Montauk Point to Bermuda. Twenty-five stations were occupied on this line. At three of these, special chemical studies of the whole water-column were undertaken and also a full program of closing net hauls. In addition, at five other stations less complete biological collections were made.

From the temperature and salinity observations the volume of the Gulf Stream can be calculated. The chemical and biological data will be used by Dr. Norris Rakestraw and Dr. George Clarke for a study of the seasonal cycle of production and decomposition that they are making in the three chief water-masses found off this coast. Since last October periodic observations for this purpose have been secured at a point about half way across the continental shelf, at the axis of the slope water (that is to say, midway between the edge of the continental shelf and the Gulf Stream) and in the Sargasso Sea, well south of the current.

Before putting in at Bermuda, a hydrographic survey was also made of the waters within a hundred-mile radius of the islands. This was the part of the work that Dr. Thompson is particularly concerned with. Not only will it be of interest to establish the prevailing current system near Bermuda, but also repeated observations in these waters by the *Culver* are expected to show up the variations in the strength of the Gulf Stream. Why this is so can be briefly explained as follows:

Because of the effect of the earth's rotation, when the current increases, sea level should rise in the western Sargasso Sea. As the current decreases in strength, on theoretical grounds mean sea level should fall. This lowering of sea level would amount to about 60 cm. in the extreme case that the Gulf Stream stopped entirely. Two methods are available to measure the possible long period changes in mean sea level. First, they can be calculated from a suitable series of subsurface temperature and salinity observations. Secondly, they can be observed on the records of a properly installed, recording tide gauge. However, the tide gauge at Bermuda could also be influenced by the local currents as well as by the variations in strength of the Gulf Stream. For this reason, it is necessary to examine carefully the circulation near Bermuda before putting too much faith in the comparatively simple tide gauge observations. In short, it is necessary to prove that the tide gauge method is sufficiently dependable for the purposes of the Gulf Stream investigation and this is the part of the co-operative program that Dr. Thompson has undertaken. If he is successful, the frequent hydrographic sections that the *Atlantis* has been running across the Gulf Stream can gradually be abandoned. It might be added that unless it is found that the current changes in strength over a period of years by more than 10%, it is improbable that it exerts a major control on fluctuations in the climate and fishery of northern Europe.

At Bermuda Dr. H. R. Seiwell joined the *Atlantis* and then carried out a two weeks' investigation of short period variations in subsurface temperature and salinity. This work was a continuation of similar studies he has made during the past two years. Not only are these short period oscillations at mid-depths a major oceanographic phenomenon, but they also have an important significance in the Gulf Stream program. As mentioned above, the ordinary temperature and salinity observations are used to determine the changes in slope of the sea surface. It has been found that at any given point the short period internal waves cause sufficient variations to affect the dynamic height, which is the technical expression for the calculation of sea level from the observed subsurface distribution of density. Therefore, it is necessary to develop an observational technique that will eliminate the effect of these relatively rapid internal oscillations from the gradual changes in sea level resulting from the long period fluctuations in the currents.

Returning to Bermuda on July 1, the *Atlantis* on two different days took out a party from the

Bermuda Biological Station to collect live material for study at the laboratory. Then on July 7 she sailed for Woods Hole. On the return trip, a month having elapsed, the observations were repeated at twenty of the stations along the northern part of the Montauk Point-Bermuda section. These stations will be revisited shortly after the middle of August and again in early October. Thus by the end of the summer chemical and biological data will be available for a complete year and a series of observations on the strength of the Gulf Stream will have been obtained over a seventeen-month period.

Early next week the *Atlantis* will set out on a two weeks' cruise for Mr. H. C. Stetson. He will visit several of the canyons at the edge of the continental shelf between New York and Chesapeake Bay. Bottom samples will be dredged and deep mud cores will be obtained to help fix the age of the sedimentary material in which these extraordinary canyons have been cut.

Immediately following this work in submarine geology, the ship will be sent on a short trip to the Gulf of Maine to test out the new electric recording current meter that Dr. E. E. Watson has been perfecting during the past year.

EMBRYOLOGY CLASS NOTES

In the several weeks since the beginning of the session on June 21, the members of the Embryology Class have studied the developmental minutia of forms from the majority of the animal phyla. In the first week, Fundulus, Cunner and Toadfish were pressed into giving their all in the interest of this new generation of embryologists; routine morphology as well as investigation along experimental lines was carried out under the direction of Dr. Goodrich. The next section of work was devoted to the Coelenterata under Dr. Ballard, who directed attention especially to a comparative study of those forms showing the modification of the medusaed generations; the incredible beauty and delicacy of the hydroids came as a distinct surprise to many of those whose introduction to the Coelenterates had been of the formal "formalin-Obelia" variety. Following these, Dr. Hamburger took charge, and, despite the scarcity of female Loligo, was able to provide the class with all of the early cleavage stages; the distressing abundance of the later developmental stages was the causative factor of many feverish hours of sketching and scanning of Watase, Brooks, and Williams. The Echinodermata were next studied under Dr. Schotté, using Arbacia and Asterias material for the most part; as a striking supplement to that portion of the work concerned with the experimental manipulation of the eggs of these forms, the Harvey microscope-centrifuge was demonstrated to the class. It was during the course of the latter demonstration that Dr. Schotté encarmined his intent visage by explaining the mechanics of the machine in a voice that had in it the elements of a lion's roar and a banshee's wail. Artemia, Balanus, Lininia, and the elusive Hippa were made available next, followed in short order by fertilization studies in the classical Nereis; cleavage was followed in Chaetopterus and Cumingia, and in Nereis by a few adventuresome souls with the patience of Job. Cell-lineage was followed out in the well-known gasteropod, Credpidula, by means of the excellently-stained "Slides by Milford"; all of the above work was carried out under the direction of that amazing little man, Dr. Whitaker. At the present time Dr. Hamburger is holding forth upon the larvae of the Annelids and Molluscs.

Many interesting divergences were taken along special lines of investigation in lectures given by the instructors in the course, largely upon their own research work. Special lectures were enjoyed at intervals, among them those by Dr. Vanderbroek, Dr. Willier and Dr. Weiss; the context of such lectures have been presented elsewhere, and need only be mentioned here. Special interest was evoked by the Packard lecture upon the "History of Embryology," and by the "Genetics and Embryology" lecture delivered by Dr. Morgan; the material presented was interesting in content and texture, as lectures given by such "grand old men" in biology invariably are.

At this point, lest the reader get the impression that the '38 crop of embryologians are made of sterner and more studious stuff than those of previous years, we hasten to gainsay such an impression by briefly delineating the extra-lab activities of the class members. Anent baseball: the deleterious effect of "K-ions" (physiology) upon the "Primitive Streaks" (embryology) was well demonstrated in a "pitched" battle one afternoon a week or so ago, said effects localizing in the respective anatomies of embryologists Woodward, Waddill and Stableford; the former suffered the most grievous hurt and the latter made the loudest moan. It was subsequently rumored that Organizer Berry became bellicose after the defeat and was told by a "K-ion" that the physiologists wanted none of his dorsal lip. Anent the picnic: the annual outing nearly came a cropper due to the contrary weather and the famous Radcliff-Taylor's faulty memory (no sausage for the Alobsterite faction and no butter for the Mytiletes); but the indomitable *Nereis* and the waddling *Winifred* breasted the waves and ensconced its damp but singing cargo at Tarpaulin Cove for a

sandy dinner; a boat trip to Oak Bluffs is planned on the money remaining from the picnic, and is tentatively scheduled for the last day of the course. Rochester Philips and Yale "Vultch" Rogoff scampered away from Dr. Whitaker's watchful eyes long enough to swim the Hole in rather choppy weather.

At the present time the bulk of the class is gathered about one of the sea-water aquaria gaping at a Portuguese Man-of-War brought up from the seas; the irridescent beast has obligingly made three fish vanish into its gasters, and has demonstrated (at least to the full satisfaction of comely Vassarite Brush) that its battery of nettle-cells are potent indeed when applied to the human skin.

—*Walter S. Milne*

BOTANY CLASS NOTES

Did you ever see a plant walking?—well, we do. Did you ever see a plant swimming?—well, we do. Did you ever dive for tight-held kelps, beneath the dashing foam and make lovely mounts until the morning, after you got home? Well, we do all that and a lot more.

"We" are the class in Algae, now in its fifth week. We have finished studying, at least formally, the Greens, the Dinoflagellates, the Diatoms, the Browns, and the Blue Greens. We are now beginning that complicated and beautiful group of the Reds. During this study the usually satisfactory method has been that of dividing up the course among the faculty members according to their specialties. Thus, Dr. Taylor discusses the Browns, and now the Reds. Dr. Drouet, the Greens, Dinoflagellates and Blue Greens, and Mr. Runk, the Charules and Diatoms. Dr. Webster gives no lectures but we are always aware of him by the great amount of material presented to us for microscopic study.

My first two questions refer to our laboratory work, where we have seen the rather surprising but efficient motion of many of the lower plants. Under the microscope we have seen much creeping and crawling and swimming among Blue Greens, Greens, Diatoms, and the disputed Dinoflagellates, whose very motion makes the zoologists covet them. One of the most satisfying of these experiences was watching the zoosporesin, *Ulva*. The tiny things turn somersaults, make jack-knife dives, and carry on marathon swimming races. They gave such a good performance that one of our enterprising Harvard men is now absorbed in a study of the cytology of *Ulva*. He can be found many afternoons diving around the red spindle, usually accompanied by several other members of the class, who think it a grand excuse for a row, and who naturally come back with a couple of pails full of fine specimens.

Somehow or other, it is difficult to convince algae to grow in pure stands out in the wilds, with the result that, except for the Browns and some Greens which are of a convincing size, we often hear such phrases as this: "Has anyone found a *Dimorphosoccus*" or "Which of these half-dozen is *Oscillatoria*." This difficulty inspired our other Harvard student to try his hand at culturing algae but it is a long, hard process.

Of course, the highlights of the weeks are the trips, for is not everyone advised to "join the Botany Class and see the world"? There is no doubt that we get around. Our first trip was the traditional one to Nobska Spring, Chara Pond, Oyster Pond, and Cedar Swamp. The last was a sort of botanical Venice. At least, the only path was composed of water three feet deep. After this, searching of the many bottles we collected ferreted out some thirty-six genera from the various ponds. We all looked forward with delightful anticipation to the first trip aboard the *Nereis*. We carefully prepared bottles and kits the night before, only to wake to a day of wind and high waves, which postponed our trip twenty-four hours. The next day, however, was a fine one, and since we rode to Cuttyhunk, our first sight of the beautiful Elizabeth Isles included them all. We landed in a cove on one side of Cuttyhunk, where the barrier beach makes a fresh water pond, and there we collected very profitably for a couple of hours. Lunch was very welcome, but did not keep us long and we soon hiked across the island to the town of Gosnold, where the *Nereis* awaited us. By this time we were much more expert, and the evening's alga-hunt brought sixty-one genera to light.

Our next trip was a hard day's work. We landed first on Nashawena, and scattered among the many fresh-water ponds and puddles among the sand dunes out behind the beach. After filling many bottles and sheets of newspaper, we embarked for Pasque. Here we walked across the island, each group by a different path, and thus covered most of the fresh water ponds this rather sear island holds. The position of one of these was pointed out by a deer who ran as we approached his watering place. The most extensive and richest of these bodies of fresh water is the Ice Pond, near which our boat was docked.

Lunch did not mean any time out, for we ate while the *Nereis* chugged along to Naushon, where we landed at the French Watering Place. After collecting along the edge of the pond a while, we took a few minutes to see the beach groves, and again started hiking. After climbing

several stone fences and scaring many sheep, we arrived at Tarpaulin Cove. Here our groups separated to different ponds, and used up every spare scrap of paper and bottle space we could find. In one pond we found an exceptionally large species of *Spiragyra* which made a very satisfactory microscope study. Our usual two hour race that evening turned up seventy-four genera.

Our first salt water collecting was on a Saturday afternoon out in the harbor. We first disturbed the terns on Pine Island, where we had our first experience with barnacles and rushing currents, and with the difficulty of distinguishing objects beneath swirling waters. We then rowed the short distance to Nonamesset and found many interesting things on the floor of its sandy north shore. Just to show what the currents were really like, we next went to the spindle, where we dived for kelp, and pursued such elusive forms as *Botryopsis* and *Nemalion*. We visited Grassy Island and then rowed back to the quiet of Eel Pond, where we collected from pilings and shore.

That night we had our first experience with mounting large forms, and it took us several hours to do it. However, the results were so satisfactory when they came out of press that we felt repaid for our late hours.

Our second salt-water trip took us back for an hour to Cuttyhunk. Here we went over to the ocean side of the island, since many algae seem to like being buffeted about, and learned how to collect between waves and how to save our collections from floating out to sea after we got them. We lunched on the way to Penikese, which of course had historical as well as botanical interest for us. After a couple of hours collecting, we again boarded the *Nereis* with pails and bottles full of a fine lot of specimens which again filled the press that night.

Thus far we have held two seminars. The first was a very informative discussion by Dr. Francis Drouet on "Taxonomic Problems in the Algae." It provoked a great deal of discussion, which was continued across the tea cups later in the evening.

Our second seminar was a synopsium on the activities of other summer laboratories. It was opened effectively by a thorough discussion of the Mountain Lake Biological Station by Mr. Runk, which he accompanied by several slides, both amusing and beautiful. Miss Madeline Pierce, of Vassar, followed with a description of Friday Harbor Oceanographic, and members of the class added notes on Solomon's Island, Isles of Shoals, and several other stations. —*Flora Fender*

PHYSIOLOGY CLASS NOTES

If the casual visitor to the Physiology Laboratory sometimes wears the startled expression of one who has strayed into a madhouse, who can blame him? For at any hour of any day of the week, seeming pandemonium is sure to reign. To the uninitiated, the clatter of assorted shakers, the slop of rheotomes, the rush of air pumps and the continual swish of running water, mingled with a constant, feverish scurrying of investigators and bored dogfish jumping from their tanks, smacks neither of rime nor reason.

But those who pause soon discover that these are but the outward signs of devilishly clever research. Those earnest creatures running away from each other holding opposite ends of a rod of melting glass may be botanists, protozoologists, embryologists, entomologists, chemists or even physicists struggling to reach the heights of the physiologists. Sober are they as they strain forward to read their manometers, galvanometers or stop-watches—twiddling the screws on their micromanipulators or studying the scratches on their smoked drums.

Not so sober were they when embarking on the good ship *Winifred* bound for Tarpaulin Cove. Fifty-three sailors, headed by Captain-owner Smith and First-mate Irving, put all thoughts of complicated gadgets aside and set out to enjoy the fine weather provided through the supplication of our special agent, Father O'Brien. After an afternoon of swimming, siestas, a baseball game and a watermelon seed-spitting contest, a few brave but fool-hardy members of the party walked to the end of Nonamesset to finish the remaining food at a picnic-supper, whereas the rest enjoyed a comfortable meal at the Mess. The "back-to-nature" group spent most of their return trip "deticking."

The party mood carried over to the baseball game with the Embryologists. The "Fast moving K-ions" representing the Physiology Class, thought by experts to have not even a soupcon of a chance against the highly touted "Primitive Streaks," crashed through to an 11-10 victory, accomplishing the deed for the first time in M.B.L. history.

Jubilant over this victory the Physiologists and others set out for a moonlight boat trip to Oak Bluffs. Despite the fact that fog replaced moonlight on the return trip, spirits remained high and song ran rampant prominent among which was "Dem Bones Gonna Rise Again." Will this become the slogan of these physiologists of tomorrow? —*I. M. and W. A.*

THE COLLECTING NET

Vol. XIII, No. 3 SATURDAY, JULY 30, 1938 Annual Subscription, $1.50 Single Copies, 30 Cents.

SKIN TRANSPLANTS BETWEEN EMBRYOS OF DIFFERENT BREEDS OF FOWL

DR. B. H. WILLIER and DR. MARY E. RAWLES

Biological Laboratories, University of Rochester

This report deals with a study of the developmental behavior of skin grafts made between embryos of genetically different breeds of fowl. We are to consider (1) the results of host-graft combinations of pigmented and unpigmented, pigmented and pigmented and unpigmented and unpigmented breeds and (2) the rôle that both donor and host play in feather characterization in the graft area.

Small pieces (1.0 x 0.5 mm. or less) of skin ectoderm (to which some mesenchyme adheres) stripped from the head of 72-hour chick embryos and transplanted to the right wing bud of hosts of the same age but of another breed, result at hatching in large areas of donor-colored down feathers covering the entire wing and often adjacent parts of the breast, back and thigh. After hatching the down feathers of such patches are gradually replaced by contour feathers having the form, rate of growth (*Continued on page 60*)

M. B. L. Calendar

TUESDAY, August 2, 8:00 P. M.

Seminar: Dr. J. P. Visscher: Some recent studies on barnacles.

Dr. E. R. Jones, Jr.: Observations on some of the Turbellaria of the eastern United States.

Dr. d'A. A. Welch: Some problems of distribution and variation in the Hawaiian tree snail Achatinella.

FRIDAY, August 5, 8:00 P. M.

Lecture: Prof. Robert Chambers: Structural aspects of cell division.

INDUCTION OF EMBRYONIC ORGANS IN REGENERATES AND NEOPLASMS

DR. OSCAR E. SCHOTTÉ

Associate Professor of Biology, Amherst College

Since transplantation experiments performed on all sorts of embryos have shown that during normal development early embryonic cells reveal only a small part of their potencies, it was necessary to consider the embryo as a superior unity which correlates a given embryonic cell with the rest of the embryo. Histological and morphological differentiations arising during development in the midst of proliferating cells of the embryo are then considered to be the outcome of a combination effect between the genetic constitution of an embryonic cell and its position in the embryo, the genetic constitution being identical for all cells of the embryo. For the vertebrate embryo in general it is now certain that the end result of development is not the sum of developmental potencies of individual cells of the embryo. Therefore, there must be in the embryo as a whole some factors —be they called organizers, fields or gradients,

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Photograph by Fred S. Howard

STAFF AND STUDENTS OF THE EMBRYOLOGY CLASS AT THE MARINE BIOLOGICAL LABORATORY

Standing, left to right: Dr. W. W. Ballard, F. S. Philips, C. M. Harold, Jr., A. A. Woodward, Dr. H. B. Goodrich, A. R. Copolo, H. F. Drury, M. V. Edds, Jr., J. Blanchard, A. L. Soderwall, F. G. Worden, L. T. Stableford, C. G. Bookhout, J. J. Milford, D. W. Dunham, Dr. O. E. Schotté, A. J. Finkel, Dr. D. M. Whitaker, S. F. Waddill, W. S. Milne, J. L. Williams, W. M. Rogoff, H. K. Fink, C. Berry, Jr., Dr. V. Hamburger, D. E. Copeland. **Second row**, seated, left to right: H. E. Taylor, A. Alley, C. W. J. Armstrong, E. L. Kurtz, M. D. Rogick, J. E. Rothermel, E. L. Klein, H. V. Brush, J. M. Spangler, M. Dobler, F. F. Stevens, M. G. Lewisohn. **Front row**: A. V. Terzian, H. N. Towle, J. G. Collier.

which produce that limitation of the potencies of embryonic cells which characterizes epigenetic development.

These limiting factors of normal development become organizing agents under experimental conditions such as can be best demonstrated by the action of Spemann's organizer—the upper blastoporal lip. Spemann's organizer, when transplanted onto another embryo, completely modifies the presumptive fate of parts of the embryo to the extent of inducing whole secondary embryos on a place where under normal conditions only ectoderm would be formed. Induction simultaneously demonstrates the totipotency of early embryonic cells and also the action of the organizing agents of development, since it reveals the differentiation of organs neither contained in the transplanted inductor nor expressed in the prospective significance of the involved group of cells.

Induction in the Spemann sense is, however, always limited to *early embryonic stages*. Transplantation on later stages of development soon reveals the progressive limitation of the totipotency of cells of early embryos. Thus, gastrula ectoderm shows unlimited potencies, but the experiments of Harrison and of his school performed on neurula and tail bud stages have revealed marked limitations in the morphogenetic potentialities of diverse regions of those embryos. Transplantations at still older embryonic stages show that cells, formerly totipotent, have acquired restricted potencies, compelling them into rigid directions of differentiation. After preliminary and transitory stages of totipotency, cells of the embryo become, so to speak, frozen in their destiny and no new change in differentiation may occur. Such cells are then said to be "determined" which conveys the meaning of a final, irreversible fate. The transplantation of organizers at those stages produces no change whatever on tissues; they pursue their own course of development.

The embryo, besides acquiring equilibrated structures, soon acquires also an equilibrated form which is not going to be modified any more. This stability of form of the adult—one of the most striking characteristics of living systems confirmed by daily observation has brought about the impression of the necessity of a permanent equilibrium of form and structure of the adult animal. It seems as if the building of form and organs were the business of embryonic development exclusively. With his set of organs received during embryonic development, the adult organism has to manage for the rest of his life. The morphogenetic flexibility which was found in the early embryonic stages is gone—we know indeed that for the majority of adults the loss of an organ is final.

The Spemann constriction experiment, at an early gastrula stage of a newt, in which under some conditions one obtains two fairly normal individuals from one embryo illustrates the prodigious flexibility of developmental processes of early stages to be found even in highly developed Vertebrates. Compare this experiment with the result of amputation of a leg in an adult frog where only wound healing processes without replacement of organs occur and the fundamental difference between the morphogenetic faculties of an embryo, as compared with an adult, becomes obvious.

These observational facts and much apparently conclusive experimental evidence have led biologists to assume the existance of a sharp distinction between embryo and adult in so far as the morphogenetic potencies of both stages are concerned.

True, there is a property of some animals called regeneration which enables them to replace by renewed growth with subsequent differentiation the loss of part of an organism. For most biologists, however, regeneration is merely another property of living organisms due to an exceptional physiological set-up which is peculiar to some groups of animals, lacking in others. Regeneration, for them, constitutes only a separate chapter of experimental biology, as we see illustrated by the fact that in most treatises on embryology regeneration is not even mentioned. It would be unfair to generalize in this respect and I hasten to add that many interesting conclusions concerning the similarity of problems involved in the field of embryology and of regeneration have been reached by a few authors—I quote particularly my own teacher, Professor E. Guyénot of Geneva and Dr. Paul Weiss. Still, it must be said that the demonstration of a thorough going parallelism between ontogenetic and regenerating processes has not yet been given.

As long as this has not been done, we will have to accept in the unrolling of life processes from its inception until death an arbitrary hiatus between embryo and the adult organism. Such an arbitrary separation of form producing processes in the embryo and in the adult has led to a distorted view of the significance of developmental

THE COLLECTING NET was entered as second-class matter July 11, 1935, at the Post Office at Woods Hole, Mass., under the Act of March 3, 1879, and was re-entered on July 23, 1938. It is devoted to the scientific work at marine biological laboratories. It is published weekly for eight weeks between July 1 and September 15 from Woods Hole, and is printed at The Darwin Press, New Bedford, Mass. Its editorial offices are situated on Main Street, Woods Hole, Mass. Between June 1 and October 1 communications should be addressed to Woods Hole, Mass.; at other times they should be directed to THE COLLECTING NET, Garrison, N. Y. Single copies, 30c; subscription, $1.50.

processes and has imposed artificial limits to research in the field of experimental morphology.

I propose to show, that in so far as developmental processes are concerned the distinction between embryo and adult should be abolished. The introduction of adequate experimental devices will demonstrate, at present, in the adult organism the existence of developmental faculties at least equal to those which, for the embryo, have been so brilliantly demonstrated by the methods of modern experimental embryology.

The gradual limitation of the potentialities of the embryo has been named "determination" and it must be said that the idea of "determination" has not been helpful in promoting the understanding of developmental processes. Several times Professor Ross G. Harrison of Yale has come out with severe criticism of the use of this word and he has suggested that the words "determined" and "determination" be "best dropped from the language of embryology, for there is no criterion for finding out when this condition is reached, if indeed it ever is." Progressive differentiation (a necessity of development) has been consistently confused with irreversible determination in spite of the warning of Harrison: "There is no way of finding out with certainty whether the particular quality which a cell seems to have is finally fixed. For there always may be new conditions not yet tested under which other potencies might be revealed."

The word "determination" will indeed become obsolete and meaningless if we try to apply it to the properties of the regenerating tissues of adult Amphibia. Under "adult" we understand the status of an animal in which the morphological and physiological correlations which make for differentiation have reached a stage of equilibrium. At any rate, the morphogenetic potentialities of cells have become extremely limited, inasmuch as, under *normal* conditions, they will not show any other differentiation than that which they have already manifested. If now we amputate a leg or a tail of such adult Urodele or of an old tadpole, we will observe that at the surface of amputation a series of transformations occur which eventually leads to reconstruction of the lost organ. We observe first cell proliferation leading to the formation of a cell hillock—the blastema. The regulation of the outer form of that blastema in which the digital invaginations will appear indicates morphogenesis in which the polar arrangements of the future organ seem to be indicated. Some time later microscopical sections will reveal definite signs of histogenesis followed by organogenesis.

The sequence in which proliferation, morphogenesis, histogenesis and organogenesis appear implies that the cells of the blastema are endowed with potentialities of progressive differentiation similar to those observed in the embryo.

It has further been shown that the regenerate, like embryonic tissues, also undergoes a period of indifferentiation during which it is unable to differentiate into complex organic structures if the connection with its own stump is disrupted. The experiments of de Giorgi (1924) who transplanted regenerates of the tail of the salamander onto the back of the same animal are an illustration to the point.

In transplanting stumpless young regenerates, regenerates with old tissue or old regenerates alone he showed that no differentiation into axial structures of the tail is possible if no old tissues are present or if the regenerate has not been submitted, for a sufficiently long time, to the influence of its stump. Therefore the differentiation of regenerates is not the outcome of its inherent potencies but a case of dependent differentiation very similar to cases observed during embryonic development. The only interpretation possible is that differentiation of regenerate is the result of the organizing action intrinsic in the morphogenetic fields of the animal.

The notion of fields, territories, and gradients has been brought up before but it is certainly due to the talent of Paul Weiss that this notion has received a clear and satisfactory formulation.

If the fate of a regenerate depends therefore on the inductive action of the organism, then it should be possible to change the ultimate fate of a regenerate if we remove a blastema early enough from the morphogenetic influence of its own field and submit it by transplantation to the action of another field. This has been shown in two experiments only, by transplanting tail regenerate into the leg field (Paul Weiss) and the reverse experiment, leg regenerate transplanted into tail field (Guyénot and O. Schotté). The dependence of the formation of morphologically well defined structures upon the organizing action of a field has been further demonstrated in Amphibia by experiments in which excision of the whole field prevents the regeneration of the expected organ: excision of tail in Triton, not followed by regeneration (O. Schotté); the same experiment performed on the snout of Triton with similar results (M. Vallette). The deflection of nerves of the brachial and sacral plexus, first performed by P. Locatelli, later perfected by E. Guyénot and Schotté, is another illustration of the fact that wherever proliferation of cell masses occurs (in this case the nerves stimulate proliferation at the surface where they emerge) the corresponding morphogenetic fields induce them to differentiate according to the potentialities of the field.

The potency of regenerates has, however, been tested only in so far as the possibility of induction between leg and tail is concerned. Tissues are

totipotent only when they are endowed with properties similar to those of early embryonic tissue. And if regenerating tissues of Urodele and Anuran Amphibia are totipotent, they should be capable of forming embryonic organs if properly submitted to the action of organizing fields.

After many other trials I chose for the test of the totipotency of regenerates the eye-lens relationship which since Warren Lewis, Spemann and Harrison is considered as a classic case of dependent development. A first test was made in collaboration with my student, Mr. Caudle to implant embryonic presumptive ectoderm of the gastrula of Urodele and Anuran into the eye of adults to test whether the adult eye, deprived of its lens, would continue to act as an organizer. The formation of well formed lens fibers at the expense of embryonic ectoderm proved that the eye of an adult, or of a large tadpole, no more capable of Wolffian regeneration, continues to act on embryonic tissues in a manner similar to the one of the embryonic eye cup.

In an experimental set-up in which young blastema is implanted into the lensless eye chamber of an adult it will be possible to test the developmental potencies of regenerating tissue by its reaction to a known morphogenetic field. If after some time a new lens is found in the eye previously deprived of its lens, and if the origin of that new lens can be determined with certainty, then it will be proved that regenerating tissues are able to undergo a new embryonic differentiation; in other words that they are totipotent.[1]

Altogether 156 operations were verified histologically. In 100 cases traces of the implant have been found on slides. Of these 100 cases thirty-two have shown lenses and lentoids, while twenty-three others have shown structural modifications within the implant. If we consider the general record of experiments on induction, this is quite a good result.

The lenses found in the eye previously deprived of its lens are really transformations at the expense of the implanted leg or tail blastema for the following reasons: Wolffian regeneration at the expense of the upper iris edge is out of the question, since in Urodele the beginning stages of this regeneration are perceptible only after 11-15 days. In Anuran tadpole, regeneration from the upper iris edge has been observed only in some exceptional cases and it takes long weeks in such cases. In our experiments fixation occured always before a possible beginning of regeneration. A further confirmation is given by direct observation of lens structures in the midst of the transplanted blastema, by the presence of cellular bridges between modified and unmodified parts of the transplant and, furthermore, by the presence of structural landmarks (blood and notochordal elements) within the neoformation.

In order to further test the lens-forming potencies of regenerates, I implanted embryonic eye-cups of Harrison's stage *25-27* in the midst of the regenerating blastema of a tail of large tadpoles and obtained the most surprising results. The eye-cups implanted in the midst of the regenerating mesenchyme not only induced the neighboring tissues to differentiate into typical lenses, but also induced in every case a complete redifferentiation of the surrounding cells, preceded by an intense proliferation.

The loose mesenchyme now becomes a dense mass of cells, numerous mitotic figures occur, and eventually the differentiation of organs spatially not connected with eyes can be observed: ear vesicles with labyrinth and mouth cavities appear in the midst of the tadpole tail. There is no doubt that ear and mouth are neoformations, as only very young eye-cups were implanted.

The probable explanation of this induced growth process and of the induction of embryonic organs is that the transplanted eye-cup induces an "eye-field" which, with time, spreads and becomes an "upper-head" field, thereby inducing the formation of an ear vesicle. These diverse fields complement each other and eventually a "lower-head" field is created producing the formation of a cavity typically lined with mouth epithelium.

In this new process of induction in regenerates far more generalized effects are obtained than in ordinary embryonic inductions produced under the influence of "organizers". There are several possible explanations of this expansion of differentiations which cannot be discussed here. But the whole process becomes a little more understandable if it is recalled that some progressive differentiations, which in the embryo are terminated in hours or in a few days here, unravel themselves in weeks because new, undifferentiated material is continuously added by proliferation and thus becomes submitted to the action of newly formed inductors.

It was important to know if the experiment could be repeated with another embryonic system, such as exists in the case of the hind brain acting on embryonic ectoderm. The transplantation of a piece of the medulla of an amphibian embryo will, as numerous experiments of Harrison and his school have shown, induce embryonic ectoderm to form ear vesicles. It was interesting to see if the relationship between medulla and otic vesicles known from embryos and similar to the eye-lens relationship already tested could be repeated in regenerating tissues. My former student Mr. Henry Emerson undertook this task first in Amherst College and during this past year at Yale

[1] Most of the histological side of this work has been done by Dr. Katherine Hummel and a paper on the induction of lenses at the expense of regenerating tissue in collaboration with her is now in press.

University where he continued this work under the direction of Ross G. Harrison. He has been entirely successful in this endeavor and the study of his cases is very convincing. Mr. Emerson transplanted into the regenerating mesenchyme of the tail of large tadpoles the medulla from late neurula stages. He observed the formation of vesicles which are diagnosed to be otic vesicles for the following reasons: similar position with normal otic vesicles just adjacent to the lateral wall of the medulla; similar shape, elliptical or pear-shaped in cross-section; and composed of a single layer of cells which are a high columnar epithelium. The blastema origin of the vesicles is shown by the fact that there was no superficial ectoderm present from which otic vesicles could arise by self-differentiation. The staining reactions of the vesicles and early stages are in all cases different from the staining of the implanted medulla and similar to that of the blastema mesenchyme. Moreover, in the cases fixed not longer than nine days after operation, the implanted medulla is loaded with yolk platelets whereas there are no yolk platelets at all in the cells of the ear vesicle.

The reported experiments leave little doubt about the capacity of regenerating blastemas of tail and leg of Urodele and Anuran to form such typical embryonic organs as lens and ear vesicles. They thus confirm the proposed hypothesis that young regenerating tissues are really totipotent. These tissues can, therefore, be compared in their properties with those of presumptive ectoderm of a young gastrula of Amphibia.

It further justifies the criticism of R. G. Harrison to use "determination" for mere progressive differentiation as against irreversible fixation of the fate of cellular elements as is too often done. This series of experiments reveals precisely one of the conditions in which entirely unsuspected properties of cells become patent: cells otherwise destined to form leg or tail organs now form typical embryonic organs. It further extends the span of embryonic life, at least in so far as freshly regenerating cells are concerned. Induction of embryonic organs is possible in adults—and this fact enlarges the frame of induction experiments previously regarded as limited to early embryonic stages. Is it possible from facts now known about properties of regenerating tissues of Amphibia to generalize regarding other groups of Vertebrates, including Mammals, and attribute to proliferating cells in general properties of indifferentiation resembling totipotency?

It seems far-fetched to formulate such an assumption, but are Mammals really so different from Amphibians in respect to their properties? Historically we can observe an interesting shifting of our conceptions concerning the "determination" of embryos in different groups of Vertebrates. The properties of induction and the totipotency of early embryonic cells has been demonstrated first for the newt egg, then for the frog, then for the chick and fish and at present we know that Dr. Toro has terminated his work at Columbia in which he demonstrates the inductibility of early mammalian embryos too (Verbal communication, paper in press). We see, therefore, in embryos of Mammals the same early indetermination followed by the same progressive differentiation of tissues which characterises all the other Vertebrates.

Concerning the cells of adult Mammals, I refer to the interesting study by Bloom on the potency of cells in the *Physiological Review* of 1937 which quotes numerous examples of cell behavior in Mammals indicating extensive potentialities of adult cells, and in his discussion he reaches the following conclusion: "Tissue culture, perhaps even more than the other phases of experimental embryology, has brought us to the conclusion that we cannot think of cellular differentiation, solely on the basis of structure (and function) of the cells being investigated. At each stage we must complement the actual picture of a cell with a testing of its potencies for further development. It is only by knowing whether a cell has or has not these potencies that we can say whether its level of specialization is permanent or temporary, reversible or irreversible." This is entirely in accord with what Professor Harrison has claimed as early as 1925.

It seems, therefore, quite legitimate to believe that adult mammalian tissues should not behave differently from what we now know about Amphibia. Yet, we cannot help thinking of Mammals as being more rigidly determined or, better, endowed with more pronounced irreversible differentiations than are the Amphibians. We heal our wounds but we do not regenerate our members and it is particularly this lack of regeneration of organs which makes us place the Mammals, from the point of view of cellular potentialities, in a different position than are the Amphibians.

We have, however, in Mammals cases of active cell proliferation which resemble very much those which we encounter at the surface of amputation of legs and tails of Amphibia. These are neoplasms. For the purpose of this research it is not necessary to discuss the question of the origin of cancerous formations as I see in them only a source of actively proliferating tissue. It is admissible to assume that such tissue might behave in a similar manner as do regenerating tissues of Amphibia. Many objections can, however, be formulated to the use of neoplasmic formations for an experiment to test the totipotencies of mammalian tissues. It is known that, in general, cancerous cells have acquired profound structural modifications. These modifications persist during trans-

plantations and also during explantations in tissue cultures which means that a given type of neoplasmic structure keeps its structural identity whether we transplant it to another animal or cultivate it in vitro. If then, cancerous cells are really "permanently altered cells" (W. Lewis) then the proposed experiment which seeks to impose new structural differentiations on cancerous cells by the use of appropriate organizers is impossible.

But it might be possible too that the structural alterations which we observe are only the end result of pathological conditions in the midst of the cancerous growth. It might be possible to assume that these cells lose their structural characteristics while undergoing division and that they become differentiated only after each successive mitosis. This would mean, therefore, that there is a period in the life of a cancerous cell during which it is endowed with properties of indifferentiation. The introduction into such a mass of proliferating cells of those factors which in the embryo produce differentiation might then impose upon it structural modifications otherwise unknown in neoplasms.[1]

The implantation of embryos or parts of embryos into growing tumors (Leaden mouse of Bar Harbor and mostly fibrosarcomatous tumor C252 were used for these experiments) was the procedure adopted after the implantation of primitive streak of six day mouse embryo revealed itself fruitless (chiefly due to the extreme smallness of the transplant in comparison with the rapidly proliferating sarcoma). The implant was left for several days in the tumor and the latter then studied histologically. As a detailed account of the experiments will be found in a paper now in process of publication, it might suffice to briefly indicate the general results. In all cases in which the implant or remnants has been found on slides a great variety of structural differentiations has been found also in the tumor. These differentiations cover a wide range of structures as we find representatives of the three germ layers: all sorts of cartilages and bones, parts of digestive tract, teeth, a variety of epithelia and also nervous ganglia and sensory vesicles. It must be said, however, that such structures appear also in normal and artificially produced teratomata and it is therefore not excluded that these formations are simply self differentiations of disintegrated parts of the embryo.

In three cases, however, it can be said that all the evidence points to the possibility of a real induction in the Spemann sense of the word. Long tubular formations surrounded by mesenchyme which has lost all the characteristic features of malignancy appear in the midst of the sarcoma. These neoformations appear near remnants of some structures difficult to diagnose but which are considered to be decomposed particles of the original implant. The comparison of these structures with embryos of the same age reveals that no similar structures can be found in embyros of the age of the implant or in older embyro of the absolute age of the implant. It is tentatively concluded that these cases have been obtained by a fortunate combination of circumstances and that they represent cases in which sarcomatous cells have been structurally modified under the action of organizing agents situated in the embryo.

The method so far used always introduces an element of uncertainty in the final results because of the danger of infiltration and differential survival of tissues of the implanted embryo. It must be concluded, in spite of the above tentatively formulated proposition, that the problem of the inductibility of cancerous tissues cannot be solved by the use of living mammalian organizer but only by the use of synthetical inducting substances implanted into growing cancerous tissues. Since this has so far not been done, we must reserve final judgment on the subject.

The material presented enables us to draw some conclusions concerning the three considered phenomena which we can characterize as developmental processes, viz: embryonic development, regeneration and neoplasmic growth. All three processes have one common feature; namely, that in each case we find morphogenetic differentiations being preceded by an intense proliferation leading to the formation of structureless cell masses.

There the similarity stops and marked differences appear: a) the *embryo* can be called a *self-differentiating morphogenetic system* which has within its own limits all that it takes to produce an organism. The cells of that system are originally totipotent, and progressive differentiation into structures occurs because of limitations of their totipotency by organizing agents situated *within* the egg. b) the (young) *Regenerate is a dependently differentiating system.* It is part of the organism, therefore not self-sufficient and must receive its differentiation from *without.* The cell masses of the blastema originally totipotent, become little by little restricted in their potencies and develop according to the nature of the field under the action of which they happen to fall. c) *Neoplasms* are here considered to be *systems without factors of differentiation.* Here proliferation prevails at the expense of differentiation. It is conceivable that the lack of factors of differentiation is precisely the reason why proliferation goes on ceaselessly. The introduction into these "aimless" cell masses of those

[1] The experiments were undertaken in collaboration with Dr. Katherine Hummel at the Jackson Memorial Laboratory of Bar Harbor, Maine (Director Dr. C. C. Little).

factors which in the embryo produce differentiation might stop proliferation and produce within the tumor teratoma-like differentiated structures.

The latter conclusion has to be accepted with extreme reserve but the evidence here given for regenerates justifies the conclusion that development is a continuous process since there is no such thing as an embryo or an adult existing as independent stages of the organism: "The organism never reaches a state of rest until it has run its course or is securely preserved in a bottle." (Ross G. Harrison, 1936). The use of known organizers by the methods of experimental embryology to test the potencies of proliferating cells of adults has opened new prospects for research which are full of promise.

(This article is based on a lecture given at the Marine Biological Laboratory on July 22.)

SKIN TRANSPLANTS BETWEEN EMBRYOS OF DIFFERENT BREEDS OF FOWL

(Continued from page 53)

(certain primaries and secondaries measured daily) and arrangement in tracts characteristic of feathers in corresponding positions in host controls, but always have the color of the donor breed. In other words, the feather formed resembles a host feather in all respects except for color or color pattern which is identical with that of the donor breed.

Host-graft combinations of S. C. White Leghorn, Rhode Island Red, Barred Plymouth Rock, F_1 embryo (of the cross Barred Plymouth Rock ♀ x Rhode Island Red ♂), Black Minorca, Buff Minorca, White Plymouth Rock, White Wyandotte and White Silkie breeds have been tested. Donor-colored feather areas have been obtained in all the various combinations of these breeds except those in which White Leghorn was donor to Barred Plymouth Rock, F_1 hybrid embryo and Rhode Island Red hosts. When skin ectoderm from embryos of this breed is grafted to Black or Buff Minorca hosts a patch of white feathers on the wing results. In all other combinations of White Leghorns with pigmented hosts no white feathers appear in the grafted area (In this connection it is important to note that White Leghorn skin ectoderm develops white, not black, feathers in grafts to the chorio-allantois of barred Plymouth Rock hosts.). Also combinations of the various white-feathered breeds have always produced white feathers, donor and host feathers being indistinguishable.

Skin ectoderm obtained from other regions (wing bud, leg bud or back) of the embryo is capable of producing a donor-colored feather area when transplanted to the wing bud base or to other sites such as the head, leg and tail. Further, if head skin is placed into the tail bud, the tail region of the chick develops donor-colored feathers. In these experiments, the host was White Leghorn and the donors Barred Plymouth Rock, Buff Minorca or F_1 hybrid embryo of the cross mentioned above.

Irrespective of its source, skin ectoderm produces on the head a small localized area of donor-colored feathers in contrast to a large, much spread out patch when placed into the wing bud, leg bud or tail bud. In all regions the shape, arrangement of the feathers and rate of growth within the graft area are identical with the feathers of a corresponding region in host control embryos or hatched chicks.

Recent experiments of Mr. Ray Watterson, working in our laboratory show that implants of a small piece of limb bud mesoderm of a Barred Rock embryo introduced into the wing bud of White Leghorn host embryos will produce a patch of donor-colored feathers having the same distribution and structure as skin ectoderm grafts produce. Upon hatching the down feathers are replaced by contour feathers having the barring pattern of donor control chicks. The entire limb bud mesoderm freed of overlying ectoderm inserted beneath the ectoderm of the host just behind the wing bud gives a stump-like process which is covered with donor-colored down feathers. Wing bud mesoderm of the White Leghorn grafted to a Barred Rock host likewise gives an extra wing stump but the feathers covering it are host colored rather than white like the donor. (See Proc. Nat. Acad. Sci., *23*: 542-546).

Lastly we are to consider the manner of origin of the donor-colored feather areas in the host. It is apparent from the data that both donor and host play a role in feather characterization within the graft area. Several lines of evidence indicate that structurally the feathers of this area are of host epidermal origin. 1) The rate of growth of the donor-colored feather is invariably that of the host. 2) The spread of the effect to include the wing and adjacent feather tracts on the breast, back and the thigh is too extensive to regard the implant of skin ectoderm as the entire source of the epidermal cells of the feather follicles. 3) An histological study of the skin ectoderm implant shows that it does not replace host epidermis of the developing wing bud but remains localized at the site of grafting. Its deeper portions become disorganized and its cells intermingle with and become indistinguishable from mesodermal cells

of the wing bud. 4) Implanting Silkie bantam skin ectoderm to Black Minorca produces structurally normal feathers of the same shape and rate of growth as those of the host, and not feathers with missing barbicels (a characteristic of Silkie feathers) as would be expected if the donor epidermis produced them. 5) Implants of limb bud mesoderm alone can produce donor-colored feathers. In this case the host epidermis undoubtedly forms the feather structure.

The interpretation is therefore reached that the feathers of the graft area are produced by the cooperation of (a) follicles made up of host epidermal cells and (b) chromatophores or diffusible humoral substances originating from the implant. In some way the color produced is controlled by the donor cells of the implant. The mechanism of this control remains for future elucidation.

(This article is based upon a seminar report given at the Marine Biological Laboratory on July 26.)

THE INNERVATION OF TRANSPLANTED LIMBS IN CHICK EMBRYOS

DR. VIKTOR HAMBURGER
Assistant Professor of Zoology, Washington University, St. Louis

The experimental analysis of the factors which are responsible for the formation of a typical nerve pattern has been greatly stimulated by the experiments of Harrison, Detwiler and their associates. Practically all the previous work has been done on the limb innervation of amphibians. My material of limb transplants in chick embryos gave, incidentally, a welcome opportunity to compare the situation in the chick with that found in the amphibians.

Transplantation of wings and hind limbs were made between 60 and 70 hours of incubation. Nerveless as well as innervated transplants were obtained. Whether or not a transplant is innervated depends on its distance from the spinal cord of the host. Dorsal transplants are innervated, but ventral transplants and those inside of the coeloma or loosely attached to the umbilical cord are nerveless. These latter undergo, nevertheless, normal morphogenesis. Nerve supply and function are obviously not among the factors necessary for their development.

One of the essential points brought out in the earliest transplantation experiments of Braus and Harrison on amphibia was the fact that nerves from any source (head, trunk or limb level of the central nervous system) would form a typical pattern within the transplanted limb. It was concluded that the factors responsible for the pattern formation reside in the limb, whereas the nerves are non-specific.

On the basis of the amphibian experiments, I suggested, several years ago, to distinguish, in the process of pattern formation, at least four successive phases, namely:

1) the traversing of the short space from the spinal cord to the basis of the limb,

2) the formation of a plexus at the basis of the limb,

3) the formation of the main pathways within the limb,

4) the establishment of specific terminations of sensory and motor fibers with their respective end organs.

The causal analysis showed for the amphibians that these four successive steps are caused by as many different factors or groups of factors which are all experimentally separable one from the other.

Detwiler was the first to show that nerves in growing from the spinal cord to the limb are directed toward it by non-specific agents intrinsic in the limb. The same holds for the chick. When a transplant is located very near a host limb, one spinal nerve is usually found to bifurcate, sending one branch into the transplant, the other into the host limb. Detwiler described the same situation in the case of Amblystoma.

A plexus was formed whenever more than one nerve entered the transplant. Such an accessory plexus may be composed of any combination of limb and trunk nerves, or of trunk nerves alone. This shows that plexus formation is caused by the configuration of the structures at the basis of the limb, and is not a specific property of certain nerves.

The hind limb plexus of the chick is divided into two parts. This situation is copied by transplants whenever the nerve supply is adequate. However, single nerves may enter the transplant and yet form a normal pattern within the transplant. This shows that plexus formation and the subsequent formation of the main pathways within the limb are not causally connected with each other.

The formation of these pathways is independent of the origin and the quantity of the ingrowing nerves. In our experiments, the number of nerves supplying a transplant varies widely; but even if the supply is very poor, a typical configuration of nerves is found within the limb. This holds for amphibians as well as for the chick. A single branch of one brachial nerve is capable of form-

ing an almost complete pattern in a transplanted wing. The same is accomplished by groups of trunk nerves. If the amount of fibers which enter is small, then at least a sciatic nerve with its major branches is formed, and the cruralis nerve omitted. A striking illustration of the non-specificity of nerves in this respect is given by a case in which a leg transplant replaced the right wing of the host. The brachial nerves formed a typical and almost complete leg pattern. The paths of the nerves must be determined by the configuration of the differentiating muscles, blood vessels, and skeletal elements.

Of interest is the *quantitative* development of these ingrowing nerves. The area which they have to innervate is obviously larger than their normal peripheral field. They respond to this increased demand by excessive growth. The amount of this hyperplasia is again correlated with the position of the transplant. The strongest nerves were found in dorsal transplants, whereas ventral transplants receive only a negligible supply. Apparently, the ventral position makes it not only difficult for the outgrowing pathfinder fibers to locate the transplant, but also interferes with the reinforcement of the first small fibers once the connection is established.

However, this hyperplasia remains within definite limits. Even if the transplant is located favorably, it is never filled to capacity with nerve fibers. Its nerve supply is in most cases but a fraction of that of a normal limb. Measurements of the diameters of the nerves between the ganglia and the plexus and a comparison with those of the left (control) side indicate that the hyperplasia rarely exceeds 50%, whereas a hyperplasia of several hundred per cent. would be necessary to accomplish a quantitatively complete saturation. The situation seems to be this: the hyperplasia is proportional to the original size of the nerve rather than to the actual demand. Apparently, two antagonistic mechanisms are at work; one, a growth stimulus exerted by the transplant; the other a growth limiting factor intrinsic in the nerve or in the central nervous system which sets an upper limit to the hyperplasia. The actual size of a nerve is the resultant of these two components.

(This article is based upon a seminar report given at the Marine Biological Laboratory on July 26).

EFFECT OF MECHANICAL STRESS ON CARTILAGE DIFFERENTIATED IN VITRO

Dr. Paul Weiss

Assistant Professor of Zoology, University of Chicago

Preliminary report on the "functional" structure of cartilage developed in tissue culture. Pigmented layer of the embryonic chick eye explanted with the mesenchymal (sclerotic) coat prior to the transformation of the latter into cartilage contracts and forces the sclerotic layer into numerous folds. These chondrify. The cells of the resulting cartilage are arranged in distinct patterns oriented along the lines in which the stresses arising from the deformation of the layer reach maximum values. This can be demonstrated by suitable rubber models. Thus, the cellular (and presumably also the fibrous) architecture of the cartilage conforms to the pattern of the mechanical lines of force. Indications are that the primary action of stress consists of the production of a definite orientation of cells and fibers in the distorted mesenchymal (pre-cartilaginous) sclerotic coat, and that this pattern is preserved and carried over into the subsequent transformation of the coat into cartilage. The "functional adaptation" of cartilage, a familiar occurrence in the organism, has thus been reproduced in vitro. (Cultures obtained with the assistance of Dr. Dorris).

(This article is based on a seminar report given at the Marine Biological Laboratory on July 26).

THE MARINE INVERTEBRATE COURSE OF THE M. B. L.

Dr. T. Hume Bissonnette

Instructor in Charge; Professor of Biology, Trinity College

This course differs from the courses on Invertebrates usually given at Universities and Colleges throughout the country in that its objectives are at least three-fold while theirs are almost entirely morphological or studies of comparative anatomy.

This course presents enough of the comparative anatomy of the invertebrates, learned by observation and dissection of the conventional forms along with much more varied materials than are possible in most colleges, to enable those who have not taken such courses to study at first hand the fundamentals of invertebrate structure. For more advanced students, it furnishes a review of this with plenty of living material. Secondly, from the study of many forms in the living and active condition, the general behavior and physiology of these forms are brought to the attention of students. Perhaps the most important part and

objective of the course is the study of animals in the field in their various habitats and associations. This enables students to learn by name and to recognize at sight many of the more common or interesting species native to the district and to become conversant with their specific characteristics and classification. This is facilitated by the use of condensed keys for rapid identification of common species belonging to the various phyla. These keys have been prepared by members of the staff of the course. This part of the course lays a foundation for the more specialized courses in Protozoology, Embryology, Physiology and Ecology.

This third part of the course is presented on field trips for which the class is divided into six teams. Each member of a team is taught to use one or more implements on each trip to aid the team in finding, identifying and learning the habits and preferred habitats of a comparatively large number of species in each region visited. Each team of nine or ten students is accompanied and directed by a different instructor on each excursion. These instructors are interested in different aspects of biology and in different groups of animals. So the method of attack upon the problems in the field, as in the laboratory, differs with different instructors. Each team comes under the influence and guidance of at least six different members of the staff on field trips and of nine in the laboratory.

The animals of the different phyla will be studied in the following order, though not all the groups here listed are listed separately on the program of the course;—Marine Protozoa, Porifera, Coelenterata, Ctenophora, Platyhelminthes, Nemertea, Nematoda, Annelida, Bryozoa, Mollusca, Arthropoda (including Limulus), Echinodermata, and the lower Chordata of the region.

Special lectures on Marine Zoology and Invertebrate Phylogeny will be given by members of the staff and, if possible, others by scientists working or visiting at the laboratory.

If weather permits, collecting and study trips will be taken to the following localities;—Lackey's Bay, the Breakwater Beach, Kettle Cove, Lagoon Pond Bridge near Vineyard Haven, Hadley Harbor, Cuttyhunk, North Falmouth and Tarpaulin Cove, where varied habitats are near enough together to make it possible to study several in a short time while the tide is low. Students will also spend half a day studying animals freshly dredged from different parts of Vineyard Sound. Students see how the dredging is done and how the animals studied are associated with each other and with the various types of sea-bottom.

There have been some changes in the staff since last summer. Dr. Olin Nelsen has resigned and his group is being taken by Dr. Sears Crowell. Dr. L. P. Sayles has also resigned and his group is being taken by Dr. A. M. Lucas of Iowa State College. Dr. W. F. Hahnert of Ohio Wesleyan is coming on as junior Instructor. The rest of the staff is the same as is shown in the Annual Announcement of the Marine Biological Laboratory.

EMBRYOLOGY CLASS NOTES

The embryology session terminated officially late Wednesday afternoon, July 27, and the Old Main Building reluctantly disgorged its lusty brood of '38 embryologians for the last time. During the final week of the course the class turned from Annelid and Mollusc larvae to a study of the cleavage and metamorphosis of various Urochords, among them Styela, Molgula, and Amaroucium; Dr. Ballard, who directed the work, had also planned to have the class study the stages of Gonionemus and of the jelly fishes, but of the vast numbers of Gonionemus once available in the Eel Pond only two pale and incredibly wan specimens were claimed; the Scyphozoa were more abundant, and the early embryological stages of Cyanea and Aurelia were studied.

During the latter days of the course, Dr. Hamburger and Dr. Ballard gave many interesting lectures as usual. Dr. Clark of the Pennsylvania Medical School was one of the guest speakers, lecturing to the class on the amazing technique which he has developed over a period of many years whereby certain adult tissues might be studied under high powers of the microscope while in situ. After the excellently illustrated lecture, which was given in the M. B. L. auditorium, Dr. and Mrs. Clark demonstrated their experimental animals to members of the class. On Tuesday, July 26, Mr. Meryl Rose, who has been recently investigating induction in Crepidula, discussed his work with the class. The final guest speaker was Dr. E. G. Conklin, who briefly described trends in modern embryology, and then described his most recent work on the lowly mollusc, Crepidula, which his studies have made famous.

Betwixt and between intervals of microscoping the elusive tunicate tadpoles, the embryologists joined the physiologists in a boat-trip to historical Penikese, where, it will be remembered, Louis Agassiz many years ago established a marine station for biological studies. The island, in the course of a hike to the Agassiz dedicatory tablet, was found to be over-run with rabbits, and densely inhabited with birds. A trip to Cuttyhunk and its Coast Guard station and a return trip through the boisterous swells off Gay Head provided a lively ending to the excursion. Later in the week the class visited Oak Bluffs "less-salubrious"dens on the proceeds of the annual picnic; the outing ended on the dock with a Virginia reel capered to the estatic strains of Chromatophore-Soderwell's harmonica. —*H. T. and H. S. M.*

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris Gorokhoff, Hazel Goodale and Garnette McClure.

Entered as second-class matter, July 11, 1935, at the U. S. Post Office at Woods Hole, Massachusetts, under the Act of March 3, 1879, and re-entered July 23, 1938.

Introducing

Dr. Pierre Grabar, Chef de Laboratoire à l'Institut Pasteur, Paris, and Rockefeller Foundation Fellow at Woods Hole.

Dr. Grabar studied at the University of Lille receiving his degree in chemical engineering. Then for two years he worked in the field of industrial chemistry, studying the production of synthetic urea from cyananide.

Then, pursuing his interest in natural science, he went to the University of Strasbourg, where he obtained his doctorate. He remained at Strasbourg for twelve years, first as Chief of a pathological chemistry laboratory, of the Medical Clinic of the University—where he devoted his time to research work on mineral substances in pathological conditions—and later as assistant in the Biochemical Institute of the Medical Faculty, of which Dr. Maurice Nicloux is the head. Here he gave part of his time to teaching chemistry in the school of dentistry.

While at Strasbourg he was granted a Rockefeller Foundation Fellowship for six months to study in London at the National Institute for Medical Research on ultra-filtration and then in the Carlsberg Laboratory, Copenhagen, where he worked with Dr. S. P. L. Soerensen on proteins and enzymes.

It was in April, 1937, that Dr. Grabar was elected to the position which he now holds in the Pasteur Institute. There he is chief of an independent laboratory where he is able to carry on the work of investigating ultra-filtration of proteins and enzymes, normal and immune serum, subjects in which he has been interested for the last six years.

In October of last year Dr. Grabar, with his wife who is an M. D., and their small daughter arrived in New York City. Under a Rockefeller Foundation Fellowship, he began work in the field of immuno-chemistry with Dr. Heidelberger at the College of Physicians and Surgeons of Columbia University. During the year Dr. Grabar spent part of his time visiting laboratories in Baltimore, Boston, Washington, D. C., Montreal, Toronto, and at the Rockefeller Institute in Princeton. Recently he delivered a lecture at the Cold Spring Harbor Symposium on "The Influence of Collodion Membrane Structure on the Ultra-filtration of Proteins."

Upon completion of his work here Dr. Grabar will go to Milwaukee where he will attend the symposium on proteins at the American Chemical Society and will then visit more laboratories before returning to Paris sometime in October.

CHORAL CLUB

The Woods Hole Choral Club opened its twelfth season auspiciously on Tuesday, July 5, with a rehearsal attended by a large and enthusiastic group of singers. Preparation was begun immediately for the Club's annual concert to be given in Woods Hole late in August.

The first rehearsal was held at the Warbasse estate on Penzance Point, where the singers were taken in automobiles when it was found that the Coast Guard Canteen, where it usually meets, was not available. The second and subsequent rehearsals were held, however, in the Canteen, directly across the street from the M.B.L. Mess Hall.

The program that has been selected for this year consists of a selection of religious and secular music. Several songs which have proved to be favorites in the past, such as Arkhangelsky's "Dusk of Night," are being repeated this summer.

Membership is still open to anyone interested in singing good music under competent direction. Previous experience is not necessary; sopranos and tenors are especially needed. Rehearsals are held at the Canteen on Tuesday after the seminar and on Thursday at eight o'clock.—*B. I. G.*

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
July 30	7:20	7:42
July 31	8:08	8:31
August 1	8:53	9:29
August 2	9:49	10:21
August 3	10:38	11:24
August 4	11:40	
August 5	12:25	12:32
August 6	1:24	1:34
August 7	2:14	2:27
August 8	3:07	3:12

In each case the current changes approximately six hours later and runs from the Sound to the Bay.

ITEMS OF INTEREST

An interesting event of yesterday was the presentation of a gold watch and chain to Mr. MacNaught, in commemmoration of his twenty-fifth anniversary as business manager of the Marine Biological Laboratory. The watch which is the gift of more than 200 friends bears the following inscription inside:

FRANK MAXON MACNAUGHT
from friends at the Marine Biological Laboratory
1913 - 1938

The course in invertebrate zoology opens to-day with an introductory lecture by Dr. T. H. Bissonnette who directs the work. This is being followed by Dr. Waterman's lecture on protozoa and then by laboratory work for the rest of the day. Fifty-three students are in the class.

DR. PETER GRAY, who has been a Rockefeller Foundation Fellow at the University of Rochester with Dr. Willier, has been appointed to the position of associate professor of biology at the University of Pittsburgh. Before assuming his duties there, he will return to the University of Edinburgh, where he will lecture in zoology until Christmas. He will take up his new position on January 1, succeeding the late Dr. H. H. Collins.

DR. C. D. WADDINGTON, lecturer in zoology at Cambridge University, now at the biological laboratory at Cold Spring Harbor, will come to Woods Hole in August. In his research work he is applying tissue culture to problems in genetics. Dr. Waddington has completed work on his intermediate textbook of genetics which will be published this fall.

DR. FRANK R. LILLIE, who left Woods Hole last Sunday to visit New York City and Chicago, returned late Thursday evening.

DR. S. O. MAST returned on Thursday from his visit with Dr. C. W. Metz who is spending the summer in Westmoreland Depot, N. H. During his trip Dr. Mast joined a group of people in climbing Haystack Mountain.

DR. MATILDE LANGE, head of the department of zoology at Wheaton College, Norton, Mass., has completed her three weeks' visit to Woods Hole.

DR. CARLES J. FISH, director of the Marine Laboratory of Rhode Island State College, drove to Woods Hole last week to visit especially Dr. Bigelow and Dr. Galtsoff. Dr. Fish is head of the department of biology at the Rhode Island State College.

The *Atlantis* set out on Thursday afternoon for a two weeks' cruise under the direction of Dr. Henry C. Stetson, who is being assisted by Dr. W. E. Scheville. Bottom samples will be dredged and deep mud cores will be obtained to help fix the age of the sedimentary material in which submarine canyons have been cut. Observations will be made along the edge of the continental shelf between New York and Chesapeake Bay.

DR. M. M. ZINNINGER, Associate Dean of the Medical College at the Johns Hopkins University, has been spending part of the summer at Woods Hole and is occupying Dr. Tashiro's cottage during the month of July. Dr. C. A. Mills, Heady Professor of Experimental Medicine at the University of Cincinnati, will occupy it for the balance of the season.

At the annual meeting of the M. B. L. Club Dr. H. B. Goodrich was elected President, Dr. Samuel E. Hill became Vice-President, and Dr. W. W. Ballard was re-elected Secretary.

DR. and MRS. F. M. SICHEL took a trip to Vermont over the week-end of July 10 where Dr. Sichel was looking after the installation of electrical equipment in the Department of Physiology of the School of Medicine of the University of Vermont.

The appointment of Mr. C. Lloyd Claff as associate in biology at Brown University was announced recently. A recent issue of the *Providence Sunday Journal* devoted almost a page to a full account of his life and work, including two large pictures of Mr. Claff working in his laboratory, and a cartoon captioned: "He discovered he could keep *Colpoda cucullus* awake by rocking it." Earlier this month Mr. Claff presented a motion picture showing at the evening seminar "Induced Excystment of *Colpoda cucullus* from its Protective Cyst by means of Laboratory-Grade Distilled Water."

DR. PAUL S. GALTSOFF, Acting Director of the United States Bureau of Fisheries Station, will spend a week between August 1 and August 6 in Havana, Cuba, attending a meeting of the American Malacological Union, before which he will present a paper on the reproduction and sex change in oysters. The trip to Havana is being made at the invitation of the Cuban Government, who asked Dr. Galtsoff to be their guest during his stay on the island. Dr. Galtsoff will confer with the Cuban authorities regarding several fisheries problems. He left Woods Hole on July 25, and will sail with members of the Union from Key West on July 31 on a boat dispatched for the purpose by the Cuban government.

BOTANY CLASS NOTES

Last Thursday the Botany class took its annual trip to Gay Head. The trip had been postponed a day to avoid stormy weather, but its effects had not been entirely lost as we soon discovered. The waves still rolled vigorously, and we waded around in them on the shore beneath the cliff, or climbed out to look over the immense piles of drift left by the storm. These were largely composed of hopelessly twisted kelp (*laminaria*) and most of us found the material fresher and easier to see in the water, even if we couldn't catch it half the time, and tumbled head first the other times. Among the many things we found was an unusual quantity of the rosy, curly-tailed *Asparagopsis*. Part of the class went sightseeing up the cliff, but all activity was soon stopped by the sight of fog which seemed to be rolling in. We scurried to the boat, but the fog stood still, and, therefore, we moved on a little way to start dredging. The swells left by the late storm rolled the boat one way and the dredge rolled it the other way. The result was many figures lying flat on all available space, and the loss of two of the hastily-consumed lunches. Our good sailors gathered together the results of our five dredgings, and we started home, much relieved to be moving in only one direction.

Because of the change in trip plans, we had had our weekly seminar the evening before. Dr. Taylor gave a well illustrated talk on his trip to the Galapagos Islands. Both boat and islands seemed to have as much human as scientific interest. Many of the algae were somewhat elusive, if present, since out there waves really *are* waves.

Our last seminar was varied. Dr. Ruth Patrick spoke on the activity of the Philadelphia Academy of Sciences, particularly in relation to the work on the microscopic division of diatoms. She gave an interesting account of this very old and active institution, where today both scientific workers and the general public can see fine collections. We finally had the opportunity to hear Dr. Rex Webster. He summarized the work he has done on the life history of *Tuomeya*, a rare and hitherto little known genus of Red Algae which likes the swift water of mountain streams.

Our last trip was to Black Rock, in New Bedford Harbor. We landed first on Sconticut Point and waded among the eel grass where the algae had drifted. We finally had a choice between a strained back or housemaid's knee according to whether we wished to lean over or kneel on the rocky bottom where the piles of debris had collected. After a rich collection of things we had not seen before, or only in rare quantities, we were rowed over to the Rock. The recession of the tide had left many rock pools which were very full of algae and sometimes very beautiful with feathery reds and blue-tipped Irish Moss (*Chondrus crispus*)*. The chief collection was of our eastern species of *Gelidium*, the genus from which agar is made. It grows on rocks washed by heavy surf, and, therefore, we had a little fun collecting it, although we were naturally told that this was tame compared with what other classes had done. After covering the shoreline and pools, we rowed or swam to the boat and devoured our long-delayed lunch on the ride home. When it had been properly fed, our class harmonica band gave us a fine concert.

It is now my painful duty to report that when this issue is read the algae class will have disbanded. Although a few of us will stay a short time to continue private research, most of us will have scattered over the country. The best we can hope is that we shall all see each other here again some day. —*Flora Fender*

* In one of these, a bottle full of fine looking green mats was collected and later carefully mounted. The only trouble was that it turned out to be paper.

NOTES FROM THE M. B. L. SOCIAL AND TENNIS CLUBS

The tournaments of the M. B. L. Tennis Club which were to have started last week have been delayed by bad weather. It is hoped that they can be played off next week. The entries in the doubles tournaments are as follows:

Mixed Doubles: M. and C. Speidel, S. Elwyn and Spinnler, Melland and Buck, F. and J. Miller, H. and R. Rugh, B. and D. Elwyn, E. and R. Carpenter, H. and R. Jones, C. and A. Burton, Musser and Schenthal, Norman and Beer.

Men's Doubles: Lancefield and Armstrong, Rugh and Ryan, Patten and Miller, Jones and Carpenter, Goodrich and Speidel, D. and A. Elwyn, Foster and Crawford, Schmidt and Burton, Algire and Schenthal, Buck and Duryee, Kidder and Burbanck.

Women's Doubles: Howell and Hiatt, Safford and Smith, S. and B. Elwyn, Sabin and Jones, Burton and Speidel.

Opening the social side of the second term at the M. B. L. an "invertebrate mixer" will be held tonight at the Clubhouse. Although its primary purpose is to give the new students at Woods Hole a chance to get acquainted, everyone whether a student or not is welcome.

The program selected for the phonograph concert which will be presented at the Clubhouse at eight o'clock Monday evening, August 1:

Symphonic Suite from "Tristram and Isolde"	*Wagner*
A group of madrigals	
Chaconne (directed by Stokowski)	*Bach*
Fourth Symphony	*Brahms*

SUPPLEMENTARY DIRECTORY FOR 1938

INVESTIGATORS

Anderson, R. L. prof. biol. Johnson C. Smith (N. C.) Rock 2.
Auringer, J. res. asst. biol. Columbia. Br 228.
Beck, Naomi grad. Chicago. Br 122C. W B.
Bien, Bettina H. grad. Wheaton. Br 110. W I.
Cohen, I. Memorial Hospital (N. Y.) Br 343.
Corson, S. A. instr. phys. New York. Br 328.
Crouse, Helen V. res. asst. Carnegie Inst. (Baltimore). Br 339.
Evans, H. grad. asst. biol. Williams. OM 26.
Fink, H. K. Princeton. Br 321.
Mullins, L. J. grad. California. OM Phys. Dr 2.
Rawles, Mary E. res. asst. biol. Rochester. Br 324.
Ray, D. T. asst. prof. biol. Johnson C. Smith (N. C.) Rock 2.
Sandow, A. asst. prof. biol. New York. Br 110.
Silber, R. grad. asst. biol. Washington (St. Louis). Br 110.
Smith, Audrey U. asst. prof. phys. Vassar. OM Phys. W E.
Taneia, Bedia grad. biom. New York. Br 305.
Walzl, E. M. instr. biol. Hopkins. Library.

INVERTEBRATE ZOOLOGY

THE STAFF

Investigation

Calkins, G. N. prof. proto. Columbia.
Conklin, E. G. prof. zool. Princeton.
Grave, C. prof. zool. Washington.
Jennings, H. S. prof. zool. Hopkins.
Lillie, F. R. prof. emb. emer. Chicago.
Mast, S. O. prof. zool. Hopkins.
McClung, C. E. prof. zool. Pennsylvania.
Morgan, T. H. dir. biol. lab. California Inst. Tech.
Parker, G. H. prof. zool. Harvard.
Wilson, E. B. prof. zool. Columbia.
Woodruff, L. L. prof. proto. Yale.

Instruction

Bissonnette, T. H. prof. biol. Trinity. in charge.
Crowell, P. S., Jr. instr. zool. Miami.
Hadley, C. E. assoc. prof. biol. N. J. State Teachers'.
Hahnert, W. F. instr. biol. Ohio Wesleyan.
Kille, F. R. asst. prof. zool. Swarthmore.
Lucas, A. M. assoc. prof. zool. Iowa State.
Matthews, S. A. asst. prof. biol. Williams.
Rankin, J. S. fel. zool. Amherst.
Waterman, A. J. asst. prof. biol. Williams.
Wightman, J. C. grad. biol. Brown.

STUDENTS

Acosta, Josefina M. Goucher. A 205.
Alexander, R. S. undergrad. asst. biol. Amherst.
Arnstein, Margery M. Simmons. WC.
Belda, W. H. grad. zool. Hopkins.
Bigler, Frances B.
Brown, H. City of New York. K 5.
Cooney, Marilyn R. Smith. K 2.
Crane, Todd undergrad. asst. biol. Wilson (Pa.) H 1.
Davis, J. O. grad. asst. zool. Missouri. Dr 5.
Deringer, Margaret K. grad. zool. Hopkins. W G.
Dobbelaar, M. E. instr. sci. Oradell H. S. (Leonia, N. J.)
Fahl, Helen Oberlin.
Ferguson, F. P. undergrad. asst. biol. Wesleyan. K 5.
Fleming, R. S. teach. biol. Greenville H. S. (Greenville, N. C.)
Fraser, L. A. undergrad. asst. biol. American. K 7.
Gale, Shirley grad. Radcliffe.
Graves, Irene Bridgewater State Teachers.
Griffiths, R. B. res. asst. biol. Princeton. Ka 24.
Haines, W. J. Wabash (Ind.)
Hall, Lydia R. grad. asst. histol. Mt. Holyoke.
Hamann, C. B. grad. asst. biol. Purdue.
Harris, Nellie R. undergrad. asst. biol. N. J. State Teachers. H 3.
Hoagland, Mary A. Swarthmore. K 2.
Jaeger, Lucena res. asst. zool. Missouri. H 2.
Jordan, Elizabeth L. Barnard. H 8.
Joseph, S. undergrad. asst. zool. De Pauw. Dr 10.
Kellogg, Margaret P. grad. Cornell. W I.
Kerrigan, Sylvia Cincinnati. H 4.
de Lisa, D. A. Union. Dr 9.
Love, Genevieve Pennsylvania Col. for Women. H 7.
McDonald, B. undergrad. asst. zool. De Pauw.
Morrison, P. R. Swarthmore.
Nadler, Evelyn R. Brooklyn.
Pierson, Mary E. grad. asst. zool. Mt. Holyoke.
Reyer, R. W. Cornell.
Rollason, H. D., Jr. Middlebury.
Root, Charlotte Smith. H 7.
Ryan, T. J. instr. biol. Boston Col.
Sackett, J. T. Pennsylvania.
Sanders, Mary E. De Pauw. W I.
Schaeffer, B. grad. zool. Columbia. Dr 7.
Schneider, Mathilda E. C. grad. zool. Illinois.
Sheehan, Eleanor L. instr. zool. New Hampshire.
Smith, R. I. Harvard. Dr 1.
Snedecor, J. Iowa State (Ames, Iowa). Dr Attic.
Sperry, R. W. grad. psych. Oberlin. Dr 1.
Taber, Elsie asst. instr. zool. Lander (S. C.)
Towle, Harriet N. grad. asst. zool. Wellesley. D 211.
Trowbridge, Carolyn F. Iowa (Iowa City).
Ward, Helen L. grad. asst. biol. Purdue. H 8.
Welch, d'A. A. asst. malacologist Bishop Museum (Honolulu).
Wells, Lorna A. grad. asst. zool. Oberlin.
Williams, Edith M. asst. biol. Elmira (N. Y.) H 4.

THE ROCKY MOUNTAIN BIOLOGICAL LABORATORY

The Rocky Mountain Biological Laboratory, near Crested Butte, Colorado, has almost twice as many students and investigators as last summer. Two-thirds of the students are graduates, coming from as far east as New York and Connecticut, and as far west as California and Washington State.

The new dining hall has just been completed, which also serves as a recreation center. A new research laboratory is also being built. Among the new investigators are Dr. V. Holt, Chico State College (California) and Dr. Lena B. Henderson, Randolph-Macon College.

—John C. Johnson, Director

The A. B. C. of Woods Hole for 1938

All Schedules Set to Daylight Saving Time — Bold Type Indicates P. M.

POST OFFICE

	Week Days	Sundays
Lobby open	7:00 to 8:00	10:00 to 5:00
Window service	8:00 to 6:00	Not open
Mail arrives	6:50, 10:45	
	3:20, 7:04	10:38
Mail leaves P. O.	6:45, 9:50	
	5:30	5:30

TELEGRAPH OFFICE

Weekdays
8:00 to 9:00
Sundays
9:00 to 11:00
4:00 to 6:00
Holidays
8:00 to 10:00
4:00 to 6:00

LIBRARY HOURS

Wednesdays and Saturdays
3:00 to 5:00
7:00 to 9:00
June 15 - September 15

RELIGIOUS SERVICES

Church of the Messiah (Episcopal)
Holy Communion, 8:00; Morning Prayer and Sermon (Holy Communion on first Sunday in the month), 11:00.

Methodist Episcopal Church
Morning Worship, 11:00. Church School, 10:00.

First Orthodox Congregational Church
Evening Service, 7:30.

St. Joseph's Roman Catholic Church
Mass: Sundays at 6:45 and 9:30. Weekdays at 7:00.

TRAIN SCHEDULE*

	Ex. Sun.	Ex. Sun.	Ex. Sun.	Daily	Sun. only**
Woods Hole	7:15	10:20	**1:00**	**6:00**	**8:10**
Boston	9:10	**12:35**	**3:00**	**8:00**	**10:10**

	Ex. Sun.	Sun. only	Sat. only	Ex. Sun.	Ex. Sat. & Sun.	Ex. Sun.
Boston	8:20	8:30	**1:00**	**1:15**	**4:00**	**4:48**
Woods Hole	10:45	10:38	**3:00**	**3:20**	**6:00**	**7:04**

* All trains stop at Falmouth.
** Also runs Labor Day.

BOAT SCHEDULE

Leaves	Daily	Daily	Weekdays	Sundays	Weekdays*	Daily†	Fri. only
New Bedford	7:00	9:30	**2:30**	**3:15**	....	**7:45**	....
Woods Hole	8:30	10:50	**3:45**	**4:45**	**7:10**	**9:00**	**9:30‡**
Oak Bluffs	9:20	11:40	**4:45**	**5:30**	....	....	**10:15**
Vineyard Haven	....	....	....	....	**7:50**	**9:45**	....
Nantucket	11:35	**2:00**	**7:00**	**7:45**	....	....	**12:15**

Leaves	Daily	Daily	Sundays§	Weekdays‖	Daily	Weekdays¶	Daily**
Nantucket	....	7:00	**2:15**	....	**2:45**	....	**5:00**
Vineyard Haven	6:10	....	....	....	....	....	....
Oak Bluffs	....	9:15	**4:15**	**12:00 M.**	**4:45**	**6:00**	**7:15**
Woods Hole	6:55	10:15	**5:05**	**12:45**	**5:35**	**6:45**	**8:00**
New B'df'd (due)	8:15	11:35	....	**2:00**	**7:00**	....	**9:25**

* Does not run Sept. 5. Discontinued Sept. 17.
† Three-quarters of an hour later on Tuesday and Thursday.
‡ Leaves 9:45 on Sept. 9-16.
§ Does not run September 4. Runs Labor Day. Discontinued after Sept. 5.
‖ Will not run Labor Day. Discontinued after Sept. 3 between Woods Hole and New Bedford, entirely after Sept. 10.
¶ Does not run Labor Day. Discontinued Sept. 17.
** Leaves Nantucket 12:00 Noon, Sept. 7, 9, 10, 12, 14, 16, 17, 19-24.

THE COLLECTING NET

Vol. XIII, No. 4 SATURDAY, AUGUST 6, 1938 Annual Subscription, $1.50 Single Copies, 30 Cents.

STUDIES ON BARNACLES

DR. PAUL VISSCHER

Professor of Biology,
Western Reserve University

The study of barnacles goes far back into history, so far in fact that the meaning of the word is somewhat obscure. In Old English it seems to mean a haunch or hind quarter, like a leg of mutton. Perhaps if goose neck barnacles grew larger this would be a proper comparison or perhaps haunches were not so large then as now.

For the benefit of those not acquainted with the marine organisms known as barnacles let me advise that these animals are more technically known as Cirripedia: that they belong to the Class of Crustacea and that they were characterized by the famous Huxley as an animal which kicks its food into its mouth by its feet.

And while I'm touching on that almost human characterization I'm reminded of a remark made from this platform some few weeks ago referring to chromatophores in fishes and crustacea, maintaining that color change was becoming a lost art in the human race. (*Continued on page* 86)

M. B. L. Calendar

TUESDAY, August 9, 8:00 P. M.

Informal Memorial of the Fiftieth Anniversary of the Founding of the Marine Biological Laboratory

Remarks by Dr. E. G. Conklin and Dr. F. R. Lillie

Presentation by the Trustees of a portrait of Charles R. Crane to the Marine Biological Laboratory.

FRIDAY, August 5, 8:00 P. M.

Lecture: Dr. L. G. Barth: Title to be announced later.

A QUANTITATIVE APPROACH TO THE SECRETION PROCESS OF THE THYROID

DR. EDUARD UHLENHUTH

Professor of Anatomy,
University of Maryland, School of Medicine

The topic of this evening's lecture is only one aspect of a long series of studies on the mechanism of secretion as observed in one particular example of secreting organs. It is neither the endocrine nature of the thyroid gland nor the specific effects of its secretion, the thyroid hormone which have formed the major subject of these studies, but rather the general laws which govern secretion processes. The structural elements of the protoplasma of the secreting cells, the mode of passage of the building stones of the secretions from the body fluids into the cells and the physical and chemical conditions under which this passage will take place, the activities of the protoplasmic elements in the transformation of these building stones into the secretion products, the mode of passage of the finished products out of the cells, the intrinsic and extrinsic factors which provide the stimuli for these processes and finally the agents

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WOODS HOLE IN HALF-TONE

First row, Prof. H. C. Bradley, supervising the Woods Hole Yacht Club water sports; Prof. Bradley in his laboratory; Dr. Frank Blair Hanson. **Second row,** Dr. and Mrs. E. C. Cole; Dr. T. H. Ruebush, winner of the 1937 tennis tournament and Dr. J. A. Miller, runner-up (Kidder); Drs. H. B. Goodrich, and O. E. Schotté. **Third row,** Dr. R. W. Weissenberg; physiologists at Tarpaulin Cove on their annual picnic (Audrey Smith); Dr. Walter Garrey in the M. B. L. auditorium. **Fourth row,** Drs. E. D. Adrian and Hans Spemann; Drs. C. Packard, V. Hamburger and H. B. Goodrich; Dr. E. B. Wilson.

which coordinate the functional activity of the gland cells with the activities of the organism as a whole, these are the elements which together make up the secretion mechanism. To study them, is a program, an ambition. Often in the course of our work, as the complexities of single problems have revealed themselves, it seems that we are moving in the opposite direction, away from our goal, rather than towards it. This evening, however, I want to present to you one part of our work which, we believe, looks hopeful.

Quantitative Method:

It is probably not too much to say that nearly every biological investigation will be greatly aided, if it becomes possible to express its results in numerical terms. In the special case before us not very much can be accomplished unless it is possible to find some quantitative measure which will express accurately the functional level of the thyroid. The physiologist is usually in a lesser predicament, in respect to finding such a procedure, than is the anatomist. The measuring of the O_2-consumption is still considered one of the most reliable procedures of obtaining information on the functional level of the thyroid gland. Nevertheless, as we see later on, the metabolism does not convey a sufficiently accurate knowledge as to what is going on within the thyroid at any given functional state.

In our search for an accurately measurable character we found that the changes in the colloid content lend themselves most admirably to such quantitative procedure. The results which we obtained from these measurements will form the subject of this evening's lecture. Before discussing them, I will make a few introductory remarks to acquaint you with the facts upon which this method rests.

Functional Cycle of Thyroid:

At the time when we started investigating the thyroid gland and its changes during the transformation of the salamander from an aquatic into a terrestrous animal, an adequate knowledge of the state of an active thyroid gland as contrasted to a dormant thyroid did not exist. It was found that during the amphibian metamorphosis the thyroid gland changes suddenly, from a relatively dormant condition, into one of eruptive activity, attaining at that time, its highest functional level during the entire life history of the animal. Nine points could be formulated, in which the thyroid at its maximum activity differs from a dormant thyroid gland.

As we shall see presently, in the thyroid gland the secretion product is deposited in visible form within little vesicles and is called the "Colloid". With respect to the effects of its activity, perhaps the most important event that happens in the activated thyroid, is the sudden escape and disappearance of this colloid from the lumen of the vesicles. In fact the entire activity of this gland consists of a cycle which is composed of two phases: (1) the storage phase, during which colloid is stored, and in which the thyroid remains inactive as far as its effect upon the physiological state of the organism is concerned; and (2) the releasing or active phase, during which the stored colloid is released into the circulation, and the effects of the thyroid hormone become apparent.

These changes in the colloid content of the gland are measurable and we were able to express them in figures. But as long as the changes could not be brought about at will and we had to wait until an animal metamorphosed, the use of this peculiarity in an analysis of the secretion process was very limited.

Experimental Activation:

Up to 1926 no means of activating the thyroid gland experimentally was known. In the course of our investigations we had been lead to believe that the thyroid gland cannot bring about the releasing phase by its own potencies, but that a special and extraneous mechanism which we called at that time the releasing factor, must exist somewhere to induce the release of the colloid. In search of this factor we found, in 1926, together with Schwartzbach, that a substance can be extracted from the anterior lobe of the hypophysis whose special business it is to induce and regulate the release of the colloid, and which we called the thyreoactivator hormone of the anterior lobe of the hypophysis.

If thyreoactivator (hereafter abbreviated T.A.) is injected, the resting thyroid gland, as we will see later on, is immediately transformed from the storage phase into the releasing phase. Two of the most conspicuous events taking place in such an activated gland are the disappearance of the colloid from the follicles and a marked increase in the height of the thyroid cells. It is now possible to introduce a definite amount of T.A. hormone and to establish, in response to it, a definite cell height and colloid content. At the same time the affinity of the colloid for histological dyes is

THE COLLECTING NET was entered as second-class matter July 11, 1935, at the Post Office at Woods Hole, Mass., under the Act of March 3, 1879, and was re-entered on July 23, 1938. It is devoted to the scientific work at marine biological laboratories. It is published weekly for eight weeks between July 1 and September 15 from Woods Hole, and is printed at The Darwin Press, New Bedford, Mass. Its editorial offices are situated on Main Street, Woods Hole, Mass. Between June 1 and October 1 communications should be addressed to Woods Hole, Mass.; at other times they should be directed to THE COLLECTING NET, Garrison, N. Y. Single copies, 30c; subscription, $1.50.

conspicuously changed. In sections fixed in Zenker's fluid, the colloid stains red in the inactive gland, while in the activated gland it stains blue.

General Occurrence of T.A. Effects:

The studies to be presented here have been performed on the very humble creature known as the Californian Newt, *Triturus torosus*, a tailed amphibian or salamander. Experimental activation of the thyroid by means of T.A., however, does not succeed in salamanders only, but may be obtained in practically every vertebrate animal. Dr. H. A. Teitelbaum succeeded in activating the thyroid gland of the eel, while Mr. J. E. Schenthal has obtained activation of the snake thyroid. The same responses to T.A. have been observed in more recent years in birds and mammals. Together with Drs. Aycock and Duckwood, I had the opportunity of examining a human thyroid, before and after T.A. injections. The injections were neither massive enough nor was it possible to continue them over a sufficiently prolonged period. Yet the colloid of the resting thyroid stained red, that of the active stained blue.

Structural Changes of the Thyroid Cells:

I would like to mention briefly some of the most essential changes which may be observed in the thyroid cells during activation of the thyroid, since they are closely correlated with the changes in colloid content, as will be pointed out later on. In the study of these structures we have availed ourselves of three different methods, the conventional histological technique, the hanging drop method and the examination of the thyroid in the living animal.

How to find the thyroid:

In this animal it is very easy to find the thyroid. Hardly much more is necessary than a ventral median longitudinal incision through the skin and mylohyoid muscle and reflecting the flaps laterally, in order to expose the thyroid. It can be seen easily with the naked eye as it lies at the point at which the lateral margins of the geniohyoid and sternohyoid muscles intersect. It has an oval shape and a reddish color. When seen under the dissecting scope its surface is uneven, and berry-like, owing to its composition of clear, roundish vesicles, the so-called follicles.

Removal of gland:

The gland may easily be removed or merely lifted off the pharyngeal wall without disturbing its blood circulation. When seen under the dissecting scope, floating in Ringer, this organ looks much like a clear, transparent glass bead. Its transparency permits the salamander thyroid to be used in studies which cannot be made in the thyroid of higher animals.

Study of living gland:

Without touching the organ or damaging its cells by slicing it, the whole organ can be placed in a hanging drop of Ringer, enclosed in a moist chamber, and studied whole, either in natural condition or stained with vital dyes, and may be examined with the highest oil immersion systems. In this condition it will stay alive, but inactive, for many hours, and all its structures, in minutest detail, can be examined.

More recently, Mr. Glenn Algire has devised equipment by means of which the thyroid of the newt can be examined within the living animal, with the blood circulating through it. Again this equipment is adjusted so that high oil immersion systems can be focused upon the minute structures of the gland and the changes of these structures can be followed.

Fixation:

It is important to point out that the observations made with these 3 techniques check perfectly. In particular I want to say that our histological method reproduces, in every essential, the living condition of the thyroid cell. In fact, it may be seen under the microscope that admitting osmic acid gradually to a fresh thyroid gland, has no other effect upon the cells but a slight browning. Otherwise all the structures remain unchanged, and even the transparency of the gland is retained.

Follicular structure:

As mentioned before, the thyroid gland is composed of little vesicles, the follicles. A method was devised, and applied recently with good success also to the human thyroid by Reinhoff, Stein and others, of dissecting the thyroid into its individual follicles, and thus make accurate counts of the number of follicles. Together with Miss Karns we dissected hundreds of glands, the smallest ones less than 0.0001 of a mm^3. The oval shape is characteristic of the isolated follicles of the resting gland of the common spotted salamander.

Each follicle represents a completely closed vesicle the wall of which consists of one single layer of epithelial cells, the thyroid cells. Within the vesicle is contained the colloid, the secretion product, while outside the capillaries are in close contact with the wall. It is this arrangement wherein the thyroid gland differs from all the other glands and which makes the interpretation of some of the changes that happen in the cells extremely difficult. It is evident from this arrangement, that the thyroid cells do not excrete their products directly into the circulation, but first into the lumen of the follicle, where they are stored. The thyroid cells must perform, in addition to the usual discharge of their secretion product at the free apical pole, a reabsorption

of the stored colloid and a re-excretion of it at the basal pole. Examining the cells at any given moment with the ordinary methods it is extremely difficult to decide in what direction the secretion products move.

Structure of Cells:

In the thyroid, completely at rest the cell plasma seems to consist in its entirety of a peculiar kind of granula. Each one of them is contained in a vacuole within which it moves around slowly. In life these granula have no color; they appear black or grayish. Except for these granula no other granula are visible. If the observer's attention is focused on the vacuoles the plasma seems to have a honey-combed structure, if it is focused at the granula it seems to have a granular structure, thus satisfying both of the once opposed theories, the theory of the foam-structure of the plasma advanced by Bütschli, and the other one maintained by Altmann, of the granular structure of protoplasma.

We do not know what these granula are, except that they are alive and are a most elementary portion of protoplasma. We have used the name "Plasmagranula" for them, which means that we know nothing about them.

They stain with Neutral Red and in fixed material with practically any common granula stains. They are therefore not mitochondria. They also stain with Janus Green, are not well preserved with fixatives containing acids, are made more easily visible and more resistant by chromation and stain with mitochondrial stains. Paradoxical as it seems, they are also mitochondria. We are therefore much inclined to doubt the specific nature of mitochondria among the granula of the protoplasma.

The behavior of these granules when studied with any one of the three methods of examination suggests that they are vitally concerned in the elaboration of the secretion products. In all our experiments on T.A. activation, it is clear that they first increase in size and possibly in number too, when injections first start.

The next thing that happens is the appearance of huge vacuoles in the cell plasma and the disappearance of the granula. These vacuoles, which are smooth-contoured in the living gland, but of irregular shape in fixed material, fill the entire cell. Again their significance is not known. At this time I cannot go into details about them. They may either be a liquid containing a proteolytic enzyme to dissolve the colloid or they may be, on the contrary, intrafollicular colloid, dissolved and then absorbed by the cells to be excreted basally into the circulation, or they may be both.

Soon the "lacunar type" of vacuoles disappears and a new process takes place. The granules have reappeared and are now conspicuous by their great variability in size. As injections are continued they are going on to enlarge until finally a stage is attained at which it is no longer possible to decide whether the largest of these granules should still be called granula or should be considered rather small droplets of colloid in progress of origin. As injections are continued, the size and number of droplets present in the cell plasma in addition to the small and large granules, increase progressively, until droplets are found as large as a nucleus. At that stage many of the droplets are found in all stages of dissolving, the resulting liquid to be excreted.

This would be briefly the story of the cell inclusions during a period of activation. Dr. Zurawsky has devised a method of estimating the degree of vacuolization and droplet formation and expressed it numerically in graphic form. Nevertheless this is a very inaccurate method of measuring thyroid activity.

Method of Measuring:

We are now ready to turn to the method which we have used to measure cell height and colloid content. Each thyroid is sectioned completely and the one section midway between the first and last section, the 50% section, is selected for measurement. At a magnification of 250 it is projected upon paper uniform in thickness and weight. The outlines of each follicle, both inner and outer ones, are drawn with a hard pencil; at the same time the cell heights are indicated. Then the latter ones are measured and expressed in millimeters. The outlined areas are cut out, resulting in a separation of colloid and epithelium, and weighed. These weights of different glands can be compared with one another.

Colloid and Epithelium:

The effect upon colloid and epithelium of daily injections of T.A. is obtained by measurements of animals which have received progressively increasing numbers of T.A. injections, one animal being killed each day during the course of the experiment. The values of colloid and epithelium as obtained directly by weighing the respective paper cut-outs, are plotted against the number of injections.

In one series, a daily dose of 50 mg. A.L. was administered. While the epithelium shows a distinct tendency to increase with increasing number of injections, the colloid, on the contrary, is decreasing. Recorded in this manner the values for both colloid and epithelium contain, in addition to the changes caused by the T.A., the changes due to chance variations in the size of the thyroid and in the sections. As we are interested only in the functional changes of the colloid, i.e. in the amounts of colloid released, we must eliminate the chance variations; such elimination is possible only by substituting for the actual weights, the

relative weight of the colloid or the proportion C/E. This proportion would inform us about the relative amounts of colloid present in each gland only if "E" would remain constant. As it changes too, the proportion C/C+E is preferable, because it minimizes the significance of "E" in the resulting quotient. While the value of this quotient is predominantly determined by the functional changes of the colloid it still contains an element of the functional change of "E". It is therefore not ideal, if we are interested in the colloid only; yet as will be seen, it does furnish an excellent index of functional level of the thyroid and is not far off from the real relative value of the colloid. The quotient C/C+E × 100 will be called the "Colloid Level" (hereafter abbreviated C.L.). We will remember that it decreases with an increase in functional level. The colloid level of a normal gland taken from an animal which had received 6 injections of Ringer was found to be 66%. A colloid level of 5% was found in a gland which had been activated by 9 daily injections of 100 mg. A.L.

Corrected Colloid Level:

An approach to the true relative value of colloid released after T.A. administration is possible. I cannot go into great detail, but will indicate briefly the method we used.

The strips of epithelium representing, in the sections, the walls of follicles, are imagined laid out into one continuous straight strip, having the shape of a rectangle. In a rectangle the area increases in the same proportion as the length of one of its sides. One side of that rectangle is represented by the cell height. This is a value which is entirely independent of chance variations in size and contains nothing but the functional changes. Using the values of cell height, a formula can be worked out which gives us the difference between the colloid before and after injection, i.e., Cn — Cb = D, this difference being expressed in percentages of the initial value of the colloid. A comparison between the C.L. and the values of the released colloid calculated by the new formula gives us results which are smaller than the values of the C.L.; in one place where the C.L. would show a release of colloid, the new value informs us that on the contrary colloid is being stored to beyond the control level. Such an exaggeration of the colloid released when the values of C.L. are used, was to be expected, as the increasing values of E result in a depression of the value C.L. Otherwise, however, the difference is not very great.

Variation of C.L. in Normal Animals:

One of the reasons why we have selected this species is the relative stability of its thyroid gland. Among the many normal thyroids we have seen, there are only a few which were not in a state of rest. But even so there exists an astonishing degree of variability of the colloid level. Among the controls available 69 animals have been recorded, with C.L. ranging between 28 and 87.

The mean of C.L. was 54 ± 0.93, with a standard deviation of ± 12 (± 22% of the mean). This material has been analyzed for a variety of factors which might have caused the high variability of the C.L., such as size and age of animals, food, temperature, season, etc. But none of them was found in any correlation to the differences in C.L. Also the sex of each of these animals is known. It is possible that there exists a relationship between the sex of the animals and the value of the C.L. in the controls. This relationship may mean that there are more females than males which have a relatively active thyroid gland. As the number of animals is very small, however, this proportion cannot offer more than a suggestion to be kept in mind during future work.

There is, however, one factor which did influence in a conspicuous manner the value of the colloid level. The animals were received from the dealers in batches. It will be seen that each batch has its characteristic colloid level and that the variability of C.L. among the animals of one batch is frequently much lower than it is among the total number of animals. For this reason care was taken that all animals of one particular experiment were selected from the same batch.

Still more clearly expressed is the difference in the C.L. of different batches and the difference in variability between the total number of animals and individual batches, when variability is expressed as range. In every one of the individual batches the range is considerably less than in the total number. We may express this relation by stating that in every one of these individual batches the C.L. seems to fluctuate in a characteristic manner; the amplitude of the fluctuations is peculiar to each batch and so is the level around which the fluctuations take place.

Functional Nature of Normal Variability:

While we have certain opinions about the nature of these differences, which we have not been able to test as yet, we must admit at present that we do not know their causes. We will perhaps be able to say a word later on about the significance of these fluctuations. At the present moment I should like to say that the difference in C.L. which exists between different batches seems to be distinctly of a functional nature and not simply what used to be termed a morphological difference. The following is the reason for this statement.

We have already mentioned that there is a definite relationship between cell height and C.L. in

the activated gland and that it is of a functional significance. Now the same relationship, though in a less pronounced degree, exists in the normal animals. In a general way, it may be said that the lower the C.L. the higher is the value of the cell height.

In view of the vast differences existing in the functional level of thyroids of different salamander species and in view of the differences in the functional level which have been found, normally and pathologically, among thyroids of the human species, it is of interest that when comparing different batches of animals of the same salamander species we can distinguish definitely between high and low functional thyroid types. Every batch above the average C.L. may be considered a low functional thyroid type, every one below it a high functional thyroid type.

Run of C.L. in Continued Injection:

We are now ready to discuss what happens to the C.L. when the thyroids are activated. This will be shown first in a series injected daily with 50 mg. A.L. Soon after the first injection the C.L. drops, which means that the stored colloid is speedily released and the follicles are emptied. After the 6th injection the C.L. reaches a bottom value which, grossly speaking, is maintained during the entire period of injections. In all further calculations only the period of injections following the attainment of the bottom value will be considered.

A more careful inspection of the "active part" of the series of injections shows, however, a considerable variation of the C.L. and more particularly we notice that the C.L., in spite of continued injections, is not kept down at the bottom value but begins to rise slowly until a new peak is attained; with a dose of 50 mg. this peak is reached at a C.L. = 33%, after which a new drop takes place to be followed again by a slow rise with a new drop ensuing. We will come back later on to the significance of these wave-like fluctuations. The cell height, on the contrary shows a rise whenever the C.L. drops; but otherwise behaves much like the C.L.

A very similar behavior was observed in experiments in which the dose was more or less than 50 mg. In one series a dose of 10 mg. was administered daily over a period of 40 days. Again the initial drop is visible and again the wave-like fluctuations are noticeable although this time the entire wave moves at a higher level, i.e. the release effected by this smaller dose is less than the one which could be produced with 50 mg. It is interesting to observe that the lowest C.L. which could be produced by this dose was not much higher than the one which could be produced by a dose 5 times as high. The difference consists rather in that after each drop the C.L. was allowed to come back to a much higher value than it was permitted to attain with a dose of 50 mg.

Effect of Dose:

With these considerations in mind we will first study the effect which the dose of T.A. has upon the C.L. The mean colloid levels of the active parts of 6 experimental series, corresponding to 100, 50, 30, 15, 10 and 5 mg. A.L. and the mean of the C.L. of the corresponding controls is plotted against dose. The higher the dose administered the lower is the colloid level. Moreover, with a dose of 50 mg. a maximum effect is obtained. Daily injections of 100 mg. do not surpass this effect.

The point corresponding to 5 mg. is higher than the point corresponding to the untreated controls. Searching for the cause of this apparent discrepancy we found that the colloid level which can be obtained does not depend only on the dose, but is conditioned also by the initial C.L., which prevailed before the injections started. For this reason it became necessary to use, instead of the C.L. themselves, the differences existing between the average normal C.L. of the series and each individual experimental C.L. Each point contains now not only the value of the experimental C.L., but also that of the control C. L. Again, however, it is true that a maximum effect is obtained with 50 mg. daily, i.e. with this dose as much colloid can be forced out of the gland as can be forced out at all.

In fact the lowest colloid level which we ever observed among approximately 500 measured thyroids, occurred in a 50 mg. series; in this animal it was only 0.98%. Our section shows almost no colloid at all. In contrast to this gland is another gland which had one of the highest colloid levels observed 87%. This shows the differences in colloid content as signified by the value of the C.L.

Variability of C.L.:

The next problem which has been much on our mind is the significance of the fluctuations observed in the injected animals. It has been pointed out previously that the C.L. varies among the controls too. Furthermore we stated that a correct representation of the effects of T.A. administration can be obtained only, if the initial colloid level prevailing before T.A. administration begins is taken into consideration. If these wave-like fluctuations of the experimental animals are merely expressing the fact that different individuals of the same series started with a thyroid of different colloid level, then the variability among the experimentals should be no greater than it is among the controls. Let us compare the variability (standard deviation ∂) of the injected animals with that among the controls in each series.

It is apparent that in each series the variability was greater among the experimentals than among the controls. Evidently the variability of the injected animals must result from an additional new element, not influencing the variability of the controls.

Beginning with a dose of 100 mg. the variability of the C.L. increases until it reaches between 15 and 10 mg., a maximum; from then on it drops steeply down to the value of the normal animals.

We interpreted this in the following manner. T.A. administration does not simply force the thyroid to release the colloid stored in the follicles. It rather starts a functional cycle which rolls off in a rhythmical manner and in which the two phases of the functional thyroid cycle, colloid release and colloid storage, alternate with one another. In spite of the continued T.A. injections the colloid is permitted, after it has been released, to be stored again. When the dose is high, the colloid is released down to a very low C.L., but storage proceeds only for a short time reaching never a very high level before the forces of the stimulus compel a new release. As the dose decreases, the lowest colloid levels that can be attained are not much higher than those attained under the influence of a high dose, as we have seen. But the levels up to which refilling of the follicles is permitted are getting increasingly higher, until the difference between lowest and highest C.L. and with it the variability of C.L., reaches a peak at 15 - 10 mg. A.L. Any dose below 10 mg., however, is insufficient to depress materially the C.L. and therefore the variability goes down again, until it finally reaches the normal level.

Obviously this interpretation would lead to the conclusion that not only the fluctuations in C.L. of the experimental thyroids, but also those of the normal glands, are of a functional significance and indicate that even normally rhythmical waves of colloid release and refilling of the follicles sweep over the thyroid gland; or in allusion to the rhythmical contractions of blood vessels so interestingly discussed recently by Dr. Irving, that the thyroid gland undergoes rhythmical contraction and expansion.

I should like to point out that closely related to these changes in the colloid content of the gland are the structural changes that we record when studying the sections of our injection series. Corresponding to the first colloid release the lacunar vacuoles make their appearance. During the following rise in C.L. the droplets develop and increase in size until corresponding to the second drop in C.L., these droplets dissolve and are replaced by vacuoles, and so forth in alternation.

Our data does not represent successive observations made on one single animal, but each point corresponds to a different individual. In the interpretation presented here we have attempted to piece together into the life story of a single individual these separate observations each of which was made on a different individual. I am fully aware that in doing so I am exposing myself to serious criticism and I would hardly have the temerity to present these views on the colloid changes if I did not feel that I had some additional evidence to support me.

Two years ago Dr. James U. Thompson made a careful study on the O_2-consumption in relation to T.A., in the same species, on which the previous studies were conducted. The measurements of metabolism were made by actually testing every day the same individual animals over a period, during which they received daily T.A. injections. The graphs obtained from these studies correspond in a most striking manner to the graphs obtained from C.L. measurements. During the first T.A. injections the metabolism rises until it reaches a peak after the 6th injection. We found that this was the time at which the C.L. drops down to a bottom value. After this rise the metabolism drops to a low level, corresponding to a period, during which the follicles are refilled and the C.L. rises. This drop in the O_2-consumption is followed by a second rise, indicating a second discharge of colloid and a drop in C.L., just as we have seen in the results obtained from C.L. measurements; and so forth. We see, then, that the two methods and the conclusions drawn from the resulting data support and explain one another.

Duration of the Efficacy of T.A. Injections:

This close correlation existing between the changes in C.L. and O_2-consumption brings me to another problem, that of the duration of the efficacy of T.A. injections.

It was Collip who discovered that the effects of his thyreotropic hormone of the A.L. of the hypophysis in mammals produces the effects of thyroid activation only for a very short time, and loses its effect approximately after about 6 to 10 injections. Similarly in the case of other hormones he found that the injected animals become refractive after a few injections. These observations were later confirmed by many investigators; Dr. Lewis in Dr. Frank Hartman's laboratory found the same behavior in mammals and man after injections of cortin.

In the case of the thyreotropic hormone Collip explained the development of a refractive state by assuming that an antihormone is produced in the injected animals. Other investigators who re-

examined this problem think that the refractiveness is caused by the proteins contained in the extracts rather than by the hormone itself; and still other observers claim that it is due to an exhaustion of the secretory vitality of the thyroid, accompanied, perhaps, by a degeneration of the epithelium.

Collip was led to his conclusions mostly by the behavior of the metabolism of his animals. Our own work indicates that in salamanders, at least, the metabolism alone is not capable of telling us the entire story.

In connection with this problem it is of interest to investigate at first, whether the depression of the colloid level in our experiments is due to the continued injection of T.A. or whether the C.L. would remain close to its bottom level even if the injections were stopped. Immediately after cessation the C.L. begins to rise in a persistent manner, without ever returning to the level to which it had been brought down by the injection previous to this period. It is evident, therefore, that in our experiments at least, the maintenance of a low colloid level must have been the work of the continued application of T.A.

In a series in which 40 daily injections of A.L. had been administered there was found a C.L. of only 9% in a thyroid removed after 39 injections.

Similar results occurred in another series where the C.L.'s were calculated for a range of 33 daily injections of 50 mg. A.L. As this series is pieced together with animals taken from different lots, each with a different C.L., the level of the colloid of the injected animals must be expressed by way of the difference between the C.L. of controls and that of the experimentals. As we have seen previously that the C.L. which may be produced by any given dose, depends not only on the dose but also on the initial C.L. It will be noticed that the last injections had hardly less effect than the first ones, and an examination of the thyroids shows that they are highly active. One of the glands of this series, after 26 injections of 50 mg. had a colloid level of 5% and all the characters of high activity.

So far, then, as the behavior of the C.L. goes, we cannot observe any lessening of the effects of T.A. as the number of injections increases. And concerning the metabolism, although it behaves somewhat similarly to that in mammals, we feel that at least in our animals it is capable of an explanation which does not require the assumption of any kind of antibodies. When we superimpose a graph obtained in our C.L. measurements upon a typical O_2-consumption graph obtained from a single individual, it is found that metabolism drops after about 6 injections in salamanders as it does in mammals. At the same time, however, the thyroid has lost all its stored colloid and has entered upon a period of manufacturing and storing new colloid. No small wonder that the metabolism cannot be maintained at a level as high as it was when all the colloid stored was released suddenly into the circulation. At about the same time at which the thyroid begins again to release the newly manufactured colloid and the C.L. drops in consequence of this renewed release, the metabolism rises again.

Obviously there is no necessity here of introducing the action of an antibody. The mere quantitative relations of the colloid seem to explain fully the changes in O_2-consumption. Beyond the available stores of thyroid hormone no effects upon metabolism can be expected. What is remarkable, however, is the fact that in the presence of T.A. which is continually pouring in the thyroid is nevertheless permitted to reverse its activity from one of release into one of storage. It might have been expected that after all the stored colloid had been excreted, the cells would begin, under the influence of T.A., to excrete the newly manufactured colloid directly into the circulation, instead of excreting it again first into the follicles. That this expectation along a line of simplification and time-saving is not fulfilled, must certainly have some very special significance. It is very strongly suggested that the colloid, in order to undergo the ripening which transforms it into a potent hormone, must first be excreted into the follicle and then reabsorbed by the cells.

It is, of course, not possible to prophesy how long the thyroid would be capable of keeping up this strenuous game of releasing and reconstructing its colloid in quick succession speeded up by the stimulus of the T.A. It is difficult to keep alive large numbers of animals exposed to massive doses of T.A. We may well imagine that beyond the number of 40 injections, the most we could obtain so far, that the thyroid might ultimately break down and no further effects might be secured from T.A. administration. But it does not seem likely that such cessation of T.A. effectiveness would be brought about by antibodies, as there is not the least indication so far of such a mechanism.

It is very well known that in amphibians immune reactions are very slightly developed and possibly this difference between amphibians and mammals accounts for the differences observed in thyroid response after T.A. administration. This latter difference may have to do also with a difference in the T.A. extracts used on our laboratory and in those of other laboratories. The future will have to decide these questions.

(This article is based on a lecture, illustrated with 37 slides, given on July 29, at the Marine Biological Laboratory.)

STUDIES ON BARNACLES

(Continued from Page 77)

In the Cirrepedia, however, we find as one of the most universal characteristics, the fact that in adult life they are all sessile. We make no prophecies, but we seem to imagine that in labor circles, at least, this character is rapidly becoming a human trait with all the sit-down strikes of recent months.

Barnacles properly may be divided into two major groups (1) the stalked forms, like Lepas and (2) the acorn barnacles like Bal Balanus. Now it is interesting to note that historically the Lepas barnacles are linked with geese. In our earliest printed treatises on Natural History, by Aldrovandi, Gessner and others we find almost universal credance in the myth that geese produce the Lepad barnacles and that these barnacles in turn give rise to the goslings which in turn migrate off in the fall as the common migratory goose of Europe (Anas bernicle), or the barnacle goose. And in our systematic literature, today, we still call the common stalked barnacle of the Mediterranean by the term, Lepas anatifera, which in translation means the one which produces the anas or goose.

So far as modern biology is concerned we must wait until 1821 when the larvae of Barnacles were first shown by Thompson to be like the larvae of Crustacea rather than like the larvae of Mollusks in which group they had previously been placed.

And it is worthy of note that today in America our recognized authority on barnacles is Dr. H. A. Pilsbry, of Philadelphia, who has spent his life as a student of the Molluscs. To most of this audience the name of Charles Darwin is not unknown but probably few of you are aware that in 1851 and 1853 he published two large monumental treatises on Barnacles on which he had spent most of his time since his return from the voyage of the Beagle in 1836. His interest in barnacles began while stranded in Lima, Peru, where he spent an unplanned but happy month collecting material on the shore. Among the molluscan shells he found some shells which were permeated with minute holes the thickness of a pin and less than 1/10 of an inch in length. He noted that under favorable conditions these holes were inhabited by a curious little animal possessing a cluster of jointed appendages.

After his return to England and after establishing his residence at Down, his curiosity drove him to ascertain what sort of an animal this was. He eventually described it as a new type of barnacle, a cirrepede, and gave it the name, *Cryptophialus minutus*—a boring barnale which digs its cave in the shell of several Molluscs found along the coast of South America.

I think it is of interest that the finding of this curious, minute animal should have so aroused Darwin's interest that as a result he spent the better part of 15 years of diligent study of barnacles.

The study of the cirripedia has advanced rapidly since the time of Darwin's work, but as Pilsbry so well words it, "His grasp of detail was so comprehensive and the language so lucid that one cannot expect to improve upon them and in the field he covered, one cannot do better than to imitate." Truly it was a masterful work and stands today as a masterpiece in the field.

My own studies have been concerned primarily with the behavior of the larvae. We have worked with the larvae of some 40 species with reference to factors determining attachment. Some are attracted by certain environmental factors and some are repelled. Most all barnacle larvae are positive to light when first hatched but all littoral forms with which we have worked are known to be negative to light at the time of attachment. Not only light but pressure and oxygen concentration have their role in determining the behavior of many forms.

Witness the remarkably constant distribution of the four most common species around here:

Balanus balanoides—the most abundant barnacle of the North Atlantic Coast is found only between tide lines.

Balanus eburneus—the ship's bottom barnacle is found almost exclusively from average low tide line to some four or six feet lower.

Balanus crenats—occurs at minimum low tide line and is most common at three fathoms but occasionally is found at depths as great as 50 fathoms.

And in contrast with this series of the genus Balanus, we find that the common form *Chthamalus fragilis* is found only above tide lives up to 10 to 12 feet above on points like Nobska where the splash and spray constantly keeps the rocks wet.

It is of some interest to question if this form which ranges up from the West Indies to Cape Cod with this as its northern limit, has been here for very many years, for neither Gould nor Verrill list it in their earlier surveys of the invertebrates of this region. Summer, Osbourn and Cole record it as not rare in 1909 and today it is found on almost every rock along our shore.

Balanus improvisus is unique in that it is found only in brackish water and I have found it in three localities in water which had no salty taste. Apparently it can live as an adult in fresh water but the larvae must moult in salt water.

Another series of barnacles is found only in association with crabs. Perhaps the most interesting of these is *Sacculina* which grows like a cancer at the expense of the gonads of its host.

In local waters a small boring barnacle *Alcippe lampas* is found in association with the hermit

crab. At Beaufort, North Carolina, a barnacle is found growing only in association with the Gorgonean corals.

All of these cases indicate selective attraction with reference to the cypid larvae.

In conclusion we may note a few specific items as follows:

1—That there are six stages in the naupleus life of the barnacle—six nauplean moults.

2—That the distribution of barnacles is determined by the behavior of larvae and subsequently by the food supply.

3—That certain chemical substances are attractive and others repellent to the cyprid stage.

4—That the adults of several species of barnacles survive in fresh water but that their larvae do not.

5—That larvae are so sensitive to changes in food supply—and perhaps chemical content of water—that there is no evidence of distribution of barnacles on bottoms of ships.

6—That it is the larval life of barnacles which controls the distribution of these forms.

(This article is based upon a seminar report given at the Marine Biological Laboratory on August 2.)

INDUCTION BY CAUTERIZATION IN THE AMPHIBIAN EGG

Dr. Arthur Cohen

Royal Society of Canada Fellow at Osborn Zoological Laboratory, Yale University

Spemann showed that by transplanting the dorsal lip of the blastopore of an amphibian gastrula into another embryo he was able to obtain a secondary embryonic axis. He spoke of the dorsal lip as an "organizer" and the secondary structures which form under its influence are called "induced" structures. In 1931 he obtained the induction of partial secondary embryonic axes by implanting dorsal lip and other material in which the cellular structure had been destroyed by crushing. Since then tissues and extracts of tissues of a large number of groups of the animal kingdom and even of plants have been implanted into the blastocoeles of early gastrulae and have led to the formation or induction of neural, chordal and muscular tissue. In an attempt to elucidate the mechanism of these inductions a number of investigators have approached the problem from the chemical point of view. Last summer Barth reported that he had obtained inductions by implanting digitonin into the blastocoele. He considered that the digitonin did not act directly, but as a cytolytic agent on the surrounding cells, and that it was these injured cells which actually liberated the inducing substances.

If this were so then it should be possible to obtain induced structures without implanting any substance but simply by injuring the inner layer of the presumptive non-neural ectoderm. By inserting a microcautery needle into the blastocoele of the early gastrula of *Rana pipiens* such an injury can be produced, and a number of eggs have been obtained showing the effect of this treatment. Characteristically a protuberance which sometimes takes the form of a tail-like or fin-like projection appears over the area under which the needle has been applied. A preliminary study of sections of some of these projections shows that induction has occurred, but as yet the relation between the extent of injury and the size and nature of the induced structures has not been determined.

Two criteria can be used to identify material of ectodermal origin in these embryos and early tadpoles; the presence of numerous pigment granules and the reduction or absence of yolk granules. The induced structures consist of ectodermal thickenings which give evidence of having been folded in from the surface as is the case with medullary plates; of tubes possessing well-defined lumina, possibly neural in nature and resembling the nerve tube at the extreme tip of the tail of a normal embryo; and in one case, of striated muscle which appears to have been formed directly from the ectoderm. In all the induced structures the presence of many pigment granules and the absence of yolk granules points clearly to derivation from the ectoderm. Moreover many of the induced structures remain either in continuity or in contact with the ectoderm. The ectodermal origin of the induced muscle is further emphasized by comparison with the normal myotome muscle of the same tadpole. In the latter case the muscle cells are still well-filled with yolk but only sparsely pigmented.

From these cases it seems that it is possible to induce secondary embryonic structures without recourse to implantation of any extraneous materials but with substances produced by the embryo itself when appropriately injured. Apparently the cells which are injured by the cautery needle liberate the inducing substances which then act on the surrounding ectoderm and so influence the course of its differentiation as to produce structures which do not normally arise from this portion of the ectoderm. The fact that inductions may be obtained simply by injury seems to raise a question as to whether many of the materials which have been implanted act directly as inducing agents or whether they act as cytolytic agents, first causing injury to the adjacent cells which then become the real source of the inducing substances. This may be especially true of substances of non-biological origin such as the carcinogenic hydrocarbons.

(This article is based upon a seminar report given at the Marine Biological Laboratory on July 26.)

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris Gorokhoff and Hazel Goodale.

Entered as second-class matter, July 11, 1935, at the U. S. Post Office at Woods Hole, Massachusetts, under the Act of March 3, 1879, and re-entered July 23, 1938.

Introducing

Peter Gray, Ph.D., D.I.C., Lecturer in Vertebrate Embryology at the University of Edinburgh; Rockefeller Foundation fellow at University of Rochester and Woods Hole, appointed Associate Professor of Biology at University of Pittsburgh beginning January 1, 1939.

Impressively surrounded by gadgets which he invents, a species of copepod which he discovered while studying the local fauna, and a fishing record of a 30 lb. catch of tautog in one afternoon, Peter Gray enjoys Woods Hole. He and Mrs. Gray plan to stay permanently in this country when they return again from Scotland in January, bringing their two-year old son with them.

Before receiving the Rockefeller fellowship which brought him to America for the first time, Dr. Gray lived for quite a while in Paris, and then studied in England. He was appointed zoologist at the Norwich Castle Museum, following in the footsteps of his grandfather, John Edward, director of the Natural History Museum in London. He later taught for seven years in Edinburgh. Studying under MacBride he obtained his first degree, a B.Sc., from the Imperial College of Science, London, doing research, unusual for an undergraduate, on the development of the amphibian kidney. Continuing this work and taking an honors degree in zoology with special reference to invertebrate embryology, he received his Ph.D. from the University of London and later the distinguished degree of D.I.C. from the Imperial College of Science. Among his other honors, he was appointed visiting research fellow in zoology at the University of Rochester last fall.

This summer, in addition to discovering *Doropygus curvatus* (a new species of copepod), Dr. Gray has been writing up results obtained during the past nine months on over one thousand hen's eggs subjected to the action of weak direct currents. These results have been negative so far as the effect of the currents is concerned. Any changes observed in the course of development of these eggs seem attributable to other causes. These experiments, however, are only preliminary controls to more detailed investigations with strong magnetic and static fields which might themselves produce such local currents.

Other publications already completed by Dr. Gray include three papers on the development of the amphibian kidney, studies on new techniques useful in anatomy and histology, and several papers on Copepods. He has also written a French grammar for science students. (He states with a smile that it sells for 3/6.)

Being a Britisher, Dr. Gray of course has his hobbies; he is a keen fisherman, a good tennis player, but prefers French cooking to any other. He is in fact, according to Mrs. Gray, an excellent cook himself. He likes America so much that he hopes to become an American citizen. The Grays will leave Woods Hole early in September, sailing for Scotland the 7th on the *Caledonia*.

—*M. F. M.*

DATE OF DEPARTURE OF INVESTIGATORS

Anderson, R. S.	July 29
Ballentine, R.	July 29
Belcher, Jane C.	July 30
Botsford, E. Frances	August 1
Budington, R. A.	August 1
Claff, C. L.	August 1
Cole, K. S.	July 16
Crouse, Helen V.	July 26
Ferguson, J. K. W.	August 1
Fisher, K. C.	July 23
Florkin, M.	July 28
Forbes, H. S.	July 20
Harvey, E. N.	July 26
Hiestand, W. A.	July 30
Hutchens, Louis M.	August 1
Kidder, G. W.	July 29
Liebman, E.	July 30
Obreshkove, V.	July 22
Pollister, A. W.	July 25
Pratt, D. M.	July 26
Ramsey, H. J.	July 30
Rawles, Mary E.	July 21
Runk, B. F. D.	July 30
Russell, Alice M.	July 14
Smith, D. C.	August 1
Sneider, Elizabeth A.	August 1
Southwick, Mildred D.	July 20
Strickland, J. C.	June 30
Taneia, Bedia	July 30
Turner, C. L.	July 11
Weinberg, V. S.	July 23
Wiersma, C. A. G.	June 9
Willey, C. H.	August 1

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
August 7	2:14	2:27
August 8	3:07	3:12
August 9	3:44	3:57
August 10	4:26	4:40
August 11	5:08	5:20
August 12	5:44	5:59
August 13	6:22	6:40

In each case the current changes approximately six hours later and runs from the Sound to the Bay.

ITEMS OF INTEREST

Dr. J. K. W. Ferguson left Woods Hole on August 1, to take up his new position as assistant professor of pharmacology at the University of Toronto. He graduated from there in medicine and spent a year in Cambridge, England, working on respiration under Dr. Roughton. Last year he was assistant professor of physiology at Ohio State University.

Dr. Mary J. Guthrie has been promoted from associate professor of zoology to full professor at the University of Missouri.

Dr. W. R. Witz has been promoted from instructor to assistant professor of biology at the University of Pittsburgh.

Dr. J. S. Rankin, Jr., last year teaching fellow at Amherst College, has been appointed instructor in biology. He is on the instructor's staff of the invertebrate zoology course at the M.B.L.

Charles Renn of the Woods Hole Oceanographic Institution left on Friday for Pensacola, and Montgomery, where he will study the engineering aspects of mosquito control in connection with his work for the International Division of Health of the Rockefeller Foundation.

Dr. Paul Dugal, instructor of zoology at the University of Montreal has just come to Woods Hole. He was at Swarthmore last year working under Dr. L. Irving.

From the Bulletin Board:

"Notice—Large sum of money rolled in elastic. Finder please return to M. B. L. Office."*

M. B. L. CLUB NOTES

Nearly two hundred biologists and their friends attended the M. B. L. club mixer last Saturday evening. Guests and members of the club mingled in conversation until soon after ten when Jay Smith and his phonograph orchestra furnished music for dancing. The party broke up at twelve o'clock.

The program selected for the phonograph concert to be presented at the Clubhouse at eight o'clock Monday evening, August 8 is as follows: Variations Symphoniques, Franck; Piano Concerto in B Flat minor, Tschaikowsky; Pohjola's Daughter, Sibelius; and Symphony No. 5, E Flat, Sibelius.

The M. B. L. Club will sponsor a ping-pong tournament on August 9. Singles for both men and women will be held.

* "The Collecting Net," too, would be glad to serve as recipient at its office on Water Street!

Dr. T. H. Bissonnette, in charge of the course in invertebrate zoology at the M. B. L., was the author of an article in *Science* for July 8 entitled "December-hatched Pheasants Lay in July on Normal Daylight".

Dr. Kenneth Fisher, assistant professor of biology at the University of Toronto and instructor in the M. B. L. physiology course, has left Woods Hole to attend the Physiological Congress in Zurich.

Under the title of his paper, "The Effect of Mechanical Stress on Cartilage Differentiated *in Vitro*," Dr. Paul Weiss was accidentally listed as assistant professor. He is an associate professor of zoology at the University of Chicago.

Drs. Patten and Miller defeated Drs. Buck and Duryee in tennis doubles before an enthusiastic group of on-lookers on Thursday afternoon. The score for the first set was 6-1; for the second, 6-3. A full account of the Tennis Club and its tournaments contributed by Dr. Roberts Rugh, last year's president, will be found on page 92.

TRUSTEE NOMINATIONS POSTED

The following notice is posted on the official bulletin board of the Marine Biological Laboratory:

August 2, 1938

Nominations are made by the Nominating Committee as follows:

For Trustee Emeritus—
H. S. Jennings

For Treasurer of the Corporation—
Lawrason Riggs, Jr.

For Clerk of the Corporation—
Philip B. Armstrong

Trustees of the Class of 1942:

E. R. Clark	M. H. Jacobs
O. C. Glaser	F. P. Knowlton
R. G. Harrison	Franz Schrader
*E. N. Harvey	B. H. Willier

For Membership of Executive Committee:

L. Irving	S. O. Mast

(signed) Caswell Grave, *chairman*, H. B. Goodrich, L. Irving, Roberts Rugh, W. R. Taylor.

* Absent from meetings of Board in 1935, 1936, 1937. The nominating committee recommends the suspension during the present meeting of the rule of the Board regarding non-attendance and eligibility to immediate re-election to the Board.

SOME BOOKS ON THE "NEW BOOK SHELF" OF THE M. B. L.

Barcroft, Joseph. The Brain and Its Environment. (1938) Yale Univ. Press. $2.00.

Carrel, Alexis and Lindbergh, Chas. A. The Culture of Organs. (1938) Hoeber.

De Beer, G. R. Development of the Vertebrate Skull. (1937) Clarendon. $10.00.

Dobzhansky, Theodosius. Genetics and the Origin of the Species. (1937) Columbia Univ. Press.

Du Nouy, P. Lecomte. Biological Time. (1937) Macmillan. $2.00.

Fraser, C. McLean. Hydroids of the Pacific Coast of Canada and the United States. (1937) Univ. of Toronto Press.

Goldschmidt, Richard. Physiological Genetics. (1938) McGraw-Hill. $4.00.

Needham, Jos. and Green, David E., Editors. Perspectives in Biochemistry. (1937) Cambridge. $4.75.

Newman, Horatio H.; Freeman, Frank N.; Holzinger, Karl J. Twins. A Study of Heredity and Environment. (1937) Univ. of Chicago Press.

Page, Irving H. Chemistry of the Brain. (1937) Thomas. $7.50.

Sayles, Leonard P. Manual for Comparative Anatomy. (1938) Macmillan. $1.60.

Scott, William B. A History of Land Mammals in the Western Hemisphere. (1937) Macmillan. $7.50.

Smith, Homer W. The Physiology of the Kidney. (1937) Oxford Univ. Press. $4.50.

Taylor, Wm. Randolph. Maine Algae of the Northeastern Coast of North America. (1937) Univ. of Mich. Press.

Vaughan, Thomas Wayland, and others. International Aspects of Oceanography. (1937) Nat'l Academy of Sciences.

Woodruff, Lorande Loss. Animal Biology. (1938) Macmillan. $3.75.

INVERTEBRATE CLASS NOTES

The spineless members of the Woods Hole community gathered early Saturday morning (July 30) with bright and eager faces and with microscopes freshly unpacked. The first thing they heard was Dr. Bissonnette's warning about sunburn and the proper apparel for the much heralded field trips.

There followed an introduction to the lowly but elusive protozoan by Dr. Waterman. The pursuit of the little beast was carried on assiduously with the aid of 'scopes, voluminous text books, vague memories of introductory zoology, and a sprinkling of native intelligence.

Our acquaintance with the fauna now native to Woods Hole was carried further when we were introduced to the members of the staff, our colleagues, and various specimens of investigators at the Invertebrate Mixer held at the M. B. L. Club on the evening of July 31. After much small talk, cookies and punch, we were given an opportunity to dance with these creatures. On the crowded floor the activity was most sociable—one never knew whom he'd bump into next.

The "Day of Rest" afforded opportunities to acquaint ourselves with the recreational facilities of the region, walks to Nobska Light, swimming and bridge or browsing at the Club. For those with pricking consciences there was always the laboratory and by the time Sunday evening rolled around there seemed to be several people afflicted in this manner. Drawings were due and activity was indicated.

Monday we were led through the maze of Porifera by Dr. Lucas. Activity progressed apace and the population in the laboratory after hours was simply appalling.

Our acquaintance with the Coelenterata was interrupted by the lengthy process of having the Invertebrate picture taken on the crowded, sunny lawn in front of the laboratory. The element of sport was introduced when several ominous creaks came from a bench crammed with trembling femininity. As everyone was looking at the birdie and the ordeal was about to end the unheeded threat of the overworked bench was carried out. Among creakings and groanings several surprised girls found themselves on a bench resting directly on the ground. The incident was good for a laugh and an abundance of good natured kidding. After a five minute wait for another bench volunteers were called for and the picture snapped in less time than it takes to tell. —*E. L. Jordan*

NOTES ON THE FORTHCOMING INTERNATIONAL CONGRESS OF GENETICS

Dr. H. J. Muller

University of Edinburgh; Member of the Committee on Organization of the Congress

The Seventh International Congress of Genetics will meet next summer in Edinburgh, Scotland, during the last week of August. It is expected that this will be a very representative gathering, so that opportunity will be afforded to many geneticists to come into direct contact with those working in related lines in distant countries, whom they had heard of only by reputation, or whom they may have last met years ago, at a time when the problems of their science had not yet assumed the aspect which they have today.

The president of the congress (as chosen by the British organizing committee) is N. I. Vavilov, famed for his work on the principles of variation and evolution of cultivated plants. Vavilov is director of the Institute of Plant Industry and of the Institute of Genetics of the Academy of Sciences of the U. S. S. R., and is a member of the latter academy and vice-president of the Lenin Academy of Agricultural Sciences of the U. S. S. R. Various other Russian geneticists have already signified their intention of participating in the

congress and it is expected that the bringing together of the scientists from the U. S. S. R. and from other countries in which important genetic researches have recently been carried out will result in a fructification of the work of all. As the congress was originally to have been held in the U. S. S. R. and considerable planning for it had already been done there, the attempt has been made to take advantage as far as possible of this preliminary work and to build further using this as the basis for the general plan of the congress.

The organizer of the congress and chairman of the committee on organization is Professor F. A. E. Crew, director of the Institute of Animal Genetics of the University of Edinburgh and president of the Genetical Society (British). According to information recently received from Dr. Crew, some 150 persons have already signified their intention of attending, but it is thought that relatively few of those who will come have as yet made their intention known. The others are, however, urged to write to Dr. Crew as soon as possible, to facilitate the making of preparations. If the matter must remain in doubt for some time yet, they should at least send notice of the possibility of their coming, and, if they intend to give a paper, should indicate what its general subject will probably be, so that it may find its proper place in the program before the latter becomes too crystallized.

A tour through Great Britain, with especial emphasis on points of interest to those in genetics and related subjects, is being arranged for the week preceding the congress, for those desiring to see the country in the company of their fellow scientists. On this, as well as at the congress itself, the attempt is being made to keep the expenses low. For those to whom the matter of expense is more important than that of luxurious accomodations, Prof. Crew is arranging to have tents put up, in the fields adjoining the Institute of Animal Genetics. This procedure was followed last summer, when the meeting of the Genetical Society (British) took place in Edinburgh, and it proved highly successful. Not only many of the younger contingent took up such quarters, and found them eminently satisfactory, but also some of the more established geneticists, such as Prof. J. B. S. Haldane, and a spirit of informal comradeliness was thereby introduced, that made the contacts much more worth while.

Every effort will be made to have the active geneticists of today who are already highly regarded for their works come to the congress and participate prominently, so as to bring to their fellow-workers elsewhere the benefit of their more recent studies. But it is also considered very important to have as many as possible of the younger workers, those from "the peripheries," etc., attend and make their own contributions. This is the chief reason why the above attempts are being made to allow for attendance at a relatively low cost. In addition to the above arrangements, Dr. Lindstrom, Secretary of the Genetics Society of America, informs us that he is making arrangements to secure a reduction of some 4% on certain transatlantic vessels, where groups of twenty-five or more are carried. Attention may also be called to the fact that the third class, in which fares are much lower than in tourist class, affords very satisfactory travelling nowadays on many vessels. And there are comparatively fast boats going directly from New York and Boston to Scotland, which would allow a saving of the journey to and from the south of Britain, for those willing to forego a tour of this kind.

The congress itself is expected to begin on Wednesday, August 23, and to last approximately a week (including the intermission on Sunday, August 27). There are to be some half dozen "plenary sessions," or symposia, which will not run concurrently with any other meetings of the congress, and which those of all branches will therefore have an opportunity to hear. These will take place in the mornings, for the most part. In the afternoons the congress will usually be split into sectional meetings, running concurrently, so as to allow as many investigators as possible to present their results in briefer form, to those more especially interested in their fields. At each sectional meeting, each day, it is planned to have a main "introductory" or "orientational" paper, somewhat longer than the rest, to give a greater coherence to the proceedings of that section. Discussion is much desired at these meetings, and will be invited, and some special discussion groups may in addition be organized on special topics, in the evenings, while, of course, those who wish to do so may take the initiative in getting up their own discussion or "round table" groups. Of great importance too will be the setting up of exhibits, which all geneticists are invited to send in advance, and about which it is desirable that prior notice should be sent to Professor Crew.

Many of the details of organization of the program, invitation of many of the main speakers, etc., still remain to be attended to, as it is difficult for those charged with this work to confer adequately while so scattered as most of them are in the summer season, but it is expected that all this will be rapidly crystallized in the early autumn. Meanwhile all who contemplate going can greatly assist in the process of organization by communicating, as above suggested, directly with Dr. Crew, Institute of Animal Genetics, West Mains Road, Edinburgh 9, and preferably also with Dr. Lindstrom, of the Genetics Society of America.

THE M. B. L. TENNIS CLUB AND ITS TOURNAMENTS

Although the tennis season is but half over, Secretary Miller reports that there are already 145 members of the M. B. L. Tennis Club, the great majority of whom are season members who are playing on the average of three hours per week. Under the direction of President George Kidder, two new clay courts were built during the winter period on the property recently donated to the Laboratory by Dr. E. B. Meigs, just behind the breakwater bath-house. Mr. A. I. Anderson, the builder of the courts, was obliged to blast away some rock to level off the ground and the recent heavy rainfall has tested (with very favorable results) the pitch of the courts for drainage. Since the let-up of the rain, all of the courts have been in constant use.

Membership in the M. B. L. Tennis Club is limited primarily to registered Laboratory workers and their immediate families. However, it is possible for non-laboratory workers to gain playing privileges under special dispensation of the Executive Committee of the Tennis Club. Membership for Laboratory workers for the balance of the season is $3.50, payable to the Secretary-Treasurer, Dr. J. A. Miller (Brick 111). Mr. Burt, the Groundskeeper, is authorized to demand membership identification of anyone using the courts at any time.

Tennis Tournaments are held at the M. B. L. annually for two reasons, first, to stimulate or encourage laboratory workers to take adequate physical exercise, and secondly, to provide several hundred workers, relatives and friends with exciting entertainment. While the majority of members do not enter the tournaments, all of them hope to be able to—at some future date. Over seventy-five people watched the men's doubles semi-finals on Wednesday, and last year 235 people watched the Junior finals.

The Tournament Committee, Mrs. C. C. Speidel and Mrs. P. B. Armstrong, have posted the drawings for the six different tournaments and have named the dates set for the semi-final and the final matches. On August 2nd one of these tournaments was concluded, namely the Women's Doubles in which Misses S. and B. Elwyn defeated Misses Safford and Smith in a three set match, the score being 6-1, 5-7, 6-0. Last year this tournament was not run because it lacked sufficient entries, but there are this year many more very good women players.

The Men's Doubles tournament has brought out some excellent tennis. On Wednesday afternoon the semi-final matches were played with the results as follows: Patten and Miller defeated Lancefield and Armstrong after the latter team was within two points of winning the match. The score was 3-6, 8-6, 8-6. A somewhat similar match followed in which Buck and Duryee defeated Schmidt and Burton 3-6,6-1,8-6. In the final set the score stood with advantage point and the score 5-4 against the winners four times while Buck was serving. This was an exceedingly close and exciting match and should mean that the Finals, to be played on Thursday, will be full of thrills. Patten and Miller are essentially net volleyers while Buck and Duryee play a driving game from the back line. The two types of play will be clearly in contrast.

The Mixed Doubles has not progressed very far. B. and D. Elwyn are to play C. and A. Burton, the winner to play Norman and Harlow in the semi-finals on August 7th. F. and J. Miller are to play Melland and Buck, the winners to play Elwyn and Spinnler in the semi-finals on August 7th. The finals are to be played on August 8th on the Mess Court and will no doubt be the finest match of its kind in the history of the Club.

The Women's Singles will bring Mrs. Burton and Miss Musser together and Miss Melland against Miss S. Elwyn in semi-final matches on August 5th. The Finals will be the following day. While these later matches will be very close and will bring a brand of (feminine) tennis not often seen here, one of the preliminary matches was very fine. Mrs. Burton out-steadied Miss Smith in a gruelling match which went to three sets, 6-3, 5-7, 6-3. There were some long rallies with hard driving and well angled shots.

In the Men's Singles matches only five have reached the semi-final brackets, namely Frank, Shaw, Spinnler, Miller and Rugh. Three others have not reached this bracket. The semi-finals are to be played August 11th, and the Finals on August 12th.

In the Junior Tournament, suggested and sponsored for the first time last year by Mrs. Lawrence Saunders, there have been only two matches. T. Jones defeated B. Kindred 6-3, 6-1 and C. Schmidt defeated M. E. Rugh 6-3, 6-0. The Finals are to be played on August 10th. In connection with this Tournament there was established last year a permanent trophy, given by Mrs. Saunders, on which is to be engraved each year the name of the winner of the Junior tournament. Miss Eunice Stunkard won this privilege last year, as well as an individual trophy, also presented by Mrs. Saunders.

There are five other permanent trophies, one for each of the tournaments listed above. Until this year there have been no individual awards aside from the engraving of the winners' names on the separate cups. However, P. Blakiston & Sons, through its representative Mr. R. F. Bowman, has contributed three silver cups to be permanently given to the individual winners of the Men's Singles and the Men's Doubles Tournaments. This donation, which is to be an annual affair, was the generous suggestion of Mr. Bowman who has taken a keen personal interest in the various activities of the M. B. L.—*R. Rugh*

THE COLLECTING NET

Vol. XIII, No. 5 SATURDAY, AUGUST 13, 1938 Annual Subscription, $1.50 Single Copies, 30 Cents.

THE CORPORATION MEETING OF THE MARINE BIOLOGICAL LABORATORY

DR. CHARLES PACKARD

Assistant Director of the Marine Biological Laboratory; The Crocker Institute of Cancer Research, Columbia University

At the fifty-first annual meeting of the Corporation, held Tuesday August 9th, no topics of special interest were discussed or acted upon. Following the usual custom. Dr. G. N. Calkins, secretary of the trustees, commented briefly on the action taken at the trustee meeting which had just adjourned. He announced that Drs. Laurence Irving and S. O. Mast were to serve on the executive committee and that nineteen new members had been elected to the corporation: Alexander A. Abramowitz, Cornell Medical College; Robert S. Anderson, Princeton; C. Lloyd Claff; Jacob Firth, Spring Valley, N. Y.; Gertrude Yonker Gottschall, Hunter College; Charles Hodge, IV, Temple University; Edmund Ruffin Jones, William and Mary College; Wendell J. S. Kreig, N. Y. University College of Medicine; Miriam Scott Lucas; Ronald Fraser MacLennan, (*Continued on page* 108)

M. B. L. Calendar

TUESDAY, August 16, 8:00 P. M.

Seminar: Dr. Donald P. Costello: Studies on fragments of centrifuged Nereis eggs.

Dr. Victor Schechter: Calcium and magnesium in relation to longevity of egg cells.

Drs. John Buck and Robert D. Boche: Some properties of living chromosomes.

Dr. Alfred M. Lucas: Some cytological studies on virus infected cells.

FRIDAY, August 19, 8:00 P. M.

Lecture: Dr. Columbus Iselin: The influence of fluctuations in the major ocean currents on the climate and fisheries.

SCIENCE AND SCIENTISTS AT THE M. B. L. FIFTY YEARS AGO

DR. E. G. CONKLIN

Trustee of the Marine Biological Laboratory Since 1897; Emeritus Professor of Biology, Princeton University

It is a great honor, I assure you, to be called upon to speak to you on this occasion and on such a theme as this. I must confess to you, to begin with, that I shall not be able to speak strictly on the topic which is announced, namely, "Science and Scientists of Fifty Years Ago", for it was 48 years ago that I came to the Marine Biological Laboratory. But it was so nearly 50 years that after all I have not changed the topic on that account.

As one of the older members of our group, you would of course expect me to dwell largely upon the earlier years, the earlier events, and those founders who started this institution. You of course know about the later events as well as I do. For that reason I shall go over what to many of you is not a twice-told tale but a many times told tale. I explained to Dr. Lillie a few minutes ago that I felt embarrassed in presenting again what

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A PHOTOGRAPH OF THE WORKERS OF THE MARINE BIOLOGICAL LABORATORY IN 1893

FRONT ROW, left to right: S. Watase, F. P. Gorham, N. R. Harrington, Ira van Giesen, J. E. Peabody, F. S. Conant, E. G. Gardiner, W. T. Councilman. SECOND ROW: H. G. Dyar, F. R. Lillie, F. J. Brockway, J. P. McMurrich, not identified, H. Randolph, K. Foot, C. O. Whitman, H. B. Merrill. THIRD ROW: W. L. Poteat, G. N. Calkins, A. P. Mathews, W. H. Dudley, E. G. Conklin, W. H. Everett, not identified, C. W. Dodge, A. D. Morrill, F. S. Lee, not identified, E. E. Bickford, C. Langenbeck, E. F. Byrnes. FOURTH ROW: A. L. Treadwell, J. Loeb, T. H. Morgan, H. Ayres, J. B. Platt, not identified, E. T. Brewster. Second and Third Window: P. A. Fish, W. M. Rankin, A. D. Mead, H. C. Bumpus, G. Gray.

I am sure must be familiar to many of you. He assures me, however, that there are always new persons in the audience who have not heard our story. So I hope those who have heard it before will permit me to tell it again.

The genesis, or what we might call the genetics of the Marine Biological Laboratory, looks to the Louis Agassiz school on the Island of Penikese, some 15 miles to the southwest of Woods Hole, as its parent. It is the "P" generation, as we say in genetics, and that institution resulted from lectures which Louis Agassiz had been giving around the country, and a letter he had published in the Boston newspapers which called attention to the need of a summer school of natural history. I think it had been his intention to have it located near Nahant; but a banker in New York, seeing the article and knowing the reputation of Louis Agassiz, wrote and offered the Island of Penikese for the purpose of this school. He also gave a certain sum of money for the starting of the school, and late in the spring, as was the case with this institution, they began putting up a very large building, as those of you who saw it in the earlier days know. The description of the opening of that laboratory is very beautifully and poetically described by John Greenleaf Whittier in a poem entitled, "The Prayer of Agassiz."

The fact is that the carpenters were at work on it until the very last minute. The laboratory was to open on Monday, and Agassiz put the question to the carpenters whether they would work on Sunday in order to finish the building. Of course Sunday was observed more in those days than it is now, especially since the biologists came to Woods Hole, but the carpenters voted to continue their work and have the building ready for the opening on Monday.

Fifty students were admitted to this Anderson School of Biology, and the enthusiasm there aroused has lasted for some 65 years. The number of distinguished biologists who were students there is a roll of honor and includes many of the leaders in the field of biology.

Louis Agassiz died in December of that same year, 1873, and the laboratory lacked a head. His son Alexander undertook the directorship of the laboratory for the summer of 1874, and at the close of that year, when the attendance was about fifty students, as it had been the previous season, he appealed to the colleges and universities of the country to find whether they wished to continue this institution. He said afterwards that he received no support whatever. They were not interested, and the Anderson School of Natural History on the Island of Penikese never had a third session. Those two sessions ended it, and yet its influence was enormous.

It was the first seaside school of natural history in the world. There were other laboratories where investigators had gone, as in the case of Kleinenberg's station on the Straits of Messina, but they were not schools for the teaching of natural history, and we must remember that the Penikese school, as well as the Annisquam one, were primarily intended for the training of teachers.

Following the suspension of the Penikese school, there was a period during which there was no school of that sort for the training of teachers. Of course the Chesapeake Zoological Laboratory was being conducted by Professor Brooks and some of his students, but rarely were others taken in. It was the feeling, especially on the part of the Women's Education Association of Boston, that there should be some place on the Eastern coast where Massachusetts teachers could get the training required to teach this subject. They persuaded Alpheus Hyatt, a former student of Louis Agassiz and at that time Curator of the Boston Society of Natural History, to take up the teaching of science teachers. He conducted a school and had a total of about 1200 enrolled in it. He wrote a number of laboratory guides for the instruction of these teachers, and the Women's Education Association persuaded him to open a summer laboratory on his own property at Annisquam. His daughter, Mrs. Alfred Mayor, has told me that the laboratory was opened in an old barn on his property, and the only boat they had was one that belonged to Professor Hyatt. They had very primitive conditions, but the Women's Education Association furnished a certain amount of glassware, tables and other furniture, and the work went on with enthusiasm. Indeed, it was growing for six years, 1880-1886. Then Professor Hyatt felt it was really getting out of his grasp and that he could not continue to handle it.

He called a meeting in March, 1887, of the Boston Society of Natural History, sent out letters of invitation to teachers of biology throughout the New England area asking those who would be interested in the establishment of a new biological laboratory to meet at the Boston Society and to formulate plans if they favored it.

The Collecting Net was entered as second-class matter July 11, 1935, at the Post Office at Woods Hole, Mass., under the Act of March 3, 1879, and was re-entered on July 23, 1938. It is devoted to the scientific work at marine biological laboratories. It is published weekly for eight weeks between July 1 and September 15 from Woods Hole, and is printed at The Darwin Press, New Bedford, Mass. Its editorial offices are situated on Main Street, Woods Hole, Mass. Between June 1 and October 1 communications should be addressed to Woods Hole, Mass.; at other times they should be directed to The Collecting Net, Garrison, N. Y. Single copies, 30c; subscription, $1.50.

Twenty-two such persons came together in March, 1887. They considered the subject and were unanimous that such a teaching laboratory was a very great desideratum, and that something should be done to establish it. They knew, of course, that they must have money to start with, and the question was how to get this money. The Women's Education Association at once offered to give all the apparatus that had been used at Annisquam. A number of persons made contributions, I think few of them exceeded $200 each, and eight professors of distinction from different parts of the United States were induced to give a series of popular lectures in Boston for the benefit of this enterprise. A price of five dollars was charged for the series. These lectures were given, as I say, by leading biologists and medical men, and a considerable sum of money was realized from that source. Then a musician donated to the new enterprise the proceeds from an original operetta which he had composed and which was performed in Boston.

They secured about $10,000 and then decided in March, 1888, that they had enough money to make a very small start. The account of this beginning that is given in the first Annual Report of the Laboratory says that they then had to decide the very serious problem as to where the laboratory should be located. There were many differences of opinion to reconcile, but finally Woods Hole was fixed upon, owing to the fact that the United States Fish Commission was in Woods Hole and that Professor Spencer Baird had established the Fish Commission there after ten years of surveying the coast from Nova Scotia down to the Chesapeake; also owing to some Yankee shrewdness as to what they might borrow from the Fish Commission. They bought a lot opposite this buiding, 78 x 128 feet. On this they started the south wing that stands there now, and the work on it went forward through the spring.

Until that time no announcement had been made as to when the laboratory was going to be opened, and they had no director. They were organized and incorporated. There were ten incorporators and seven trustees elected by the Corporation, as is done now, and those seven trustees, all of them now gone (the last one, Miss Susan Minns, died only last week in her ninety-ninth year) and the incorporators, all of whom, with a single exception, are no longer living, got out an announcement and tried to induce one person after another to accept the directorship. Finally they asked Professor C. O. Whitman. Now Whitman is really the father of this Laboratory, in spite of the fact that it was started by the Women's Education Association and the Boston Society of Natural History. Whitman was then the Director of the Lake Laboratory at Milwaukee, Wisconsin, which had been established by Edward Phelps Allis; only the year before, 1887, with the financial aid of Mr. Allis he had established the *Journal of Morphology*, which was really one of the finest journals of the science of biology in the world. Undoubtedly, his work at the Lake Laboratory and his establishment of the journal had much to do with his selection for the directorship of this laboratory. The Laboratory was to open on the 17th of July, 1888, and I can not do better than to read you the account which Miss Cornelia Clapp, one whose memory in this place is forever blessed, has written of the opening of the Laboratory. She was a student of Whitman's at Penikese, and as soon as she heard that Whitman was to be director she decided to come to the Laboratory.

She writes:

I had seen the circular announcing the opening of the Laboratory. I was sure that I wanted to see what it was like and to enjoy the advantages of study at the seashore. My memories of the Penikese School, to which I went in 1874 quickened my desires and the name of Dr. C. O. Whitman as director added to my interest since he also was a Penikesian.

Thus it was that I arrived at Woods Hole July 10, 1888. I made my way to the building which was to be the laboratory. It was still unfinished. Carpenters were at work making tables, putting up shelves, and doing other necessary last things before it could be occupied for work.

The first man I met was Mr. Bowles, one of the carpenters. From him and from Mr. Van Vleck, a fellow Penikesian whom I met on the street, I learned that Dr. Whitman had not arrived; that he was delayed by illness in his family; that the equipment for the building was still on the road, probably sidetracked somewhere; that it might be some time before the laboratory was opened; that no arrangements had been made for boarding, and that I must look out for myself.

So the search for a boarding place began. It proved fruitless, for Woods Hole people took no boarders. Fortunately some did take lodgers and there was an eating place at the railroad station. The eating room proved to be a dark, dingy hole where two or three men, who were working at the Fish Commission, took their meals, and there I took my first meals. I found a room temporarily at Mrs. Hatch's house across the railroad bridge, and about this time I heard of a Miss Harris, a student from Wellesley, who had been at Woods Hole and was expected to return in a day or two. A little later Miss Harris and I took rooms at Mrs. Samson's and meals at the railroad station which we gladly left when Gardiner Cottage was opened.

This cottage was placed at the disposal of the Laboratory by Mr. Fay. The Marine Biological Laboratory workers took their meals there. Dr. Gardiner sat at the head of the table and often entertained us with accounts of his life in Leipsig. This was the "Mess", so-called from the first. The name originated with Dr. Gardiner. I have very pleasant memories of the table talk and of the associations there. Later I had a room in the cottage for some time.

The laboratory building was becoming more usable every day. It was set down among boulders and the

area across the street was strewn with rocks of all sizes through which we made our winding way to the main street near the Stone Building. The way was plain enough by day but it was sometimes a little intricate and interesting when the shades of evening had fallen.

The great lack was the absence of the equipment. Now the Marine Biological Laboratory was an outgrowth of Annisquam Laboratory, which, through the kindness of Professor Hyatt, had been maintained by the Woman's Education Association of Boston from 1880 to 1886. This Association became the instigator of the movement to found a permanent biological station at Woods Hole. The women trustees were those who had been active in the project of a marine laboratory for teachers; one of these, Miss Florence Cushing, was prominent and in many ways efficient. Thus Woods Hole inherited some equipment from Annisquam but not much.

I very distinctly recollect the day when the belated freight car brought our longed-for equipment. Dr. Whitman, Dr. Minot, and I, with the assistance of Dr. William Patten of the Fish Commission, unpacked boxes and barrels of glassware and instruments. It was late in the evening when the last barrel was opened and its contents checked. Then from our arduous labors we repaired to Tommy Howes' ice cream parlor which was just closing for the night and regaled ourselves with ice cream and sherbert.

The Fish Commission was a great advantage to the Laboratory; how great one who was not present that first summer can hardly realize. The Laboratory had no boats, no nets or other apparatus required for furnishing material for study, but the Fish Commission had and we enjoyed the benefits thereof. The Fish Commission supplied the seawater for the aquaria.

The men from the Fish Commission used to come over evenings to visit the new laboratory and perhaps to consult our books, for we had in the corner of the upstairs laboratory a few shelves containing the nucleus of our present library. This consisted of some books given by Mrs. Glendower Evans, the sister of Dr. Gardiner. And Dr. Minot, I remember, was much interested in the cataloguing and arranging of these books.

That first year there was neither Wilson, nor Morgan, nor Lillie nor Conklin; they came later. At the Fish Commission were Dr. Watase, Dr. Ryder and Professor Patten.

At the Marine Biological Laboratory in the Department of Investigation were: Dr. E. G. Gardiner, Institute of Technology; Miss O'Grady, Bryn Mawr; Miss C. M. Clapp, Mount Holyoke; E. O. Jordan, Institute of Technology; Miss Helen Torrey Harris, Wellesley; Miss Isabel Mulford (Botany), Vassar; Mr. Washburn, University of Michigan.

Professor Sedgwick and his wife were staying at the hotel at Quisset Harbor and during the summer Professor Sedgwick came into the laboratory almost every day. He was a trustee and had been most influential in starting the Laboratory. I remember that he gave us a talk on the sundew, a plant which he discovered grew plentifully in the region.

The address at the opening of the Laboratory was given July 17, 1888, by Dr. Whitman, the Director. It may be found in the Report to the Trustees for 1888.

As soon as I was located and the Laboratory was opened I had to decide what work I should begin. The question was, should I enter as a student or as an investigator. I think the views of Dr. Whitman in regard to the way to study really settled the matter and I became an investigator.

The next question was, what subject should I investigate. The recently published work of Allis on Amia, done at the Allis Laboratory, had led him to consider the further study of that subject desirable. So he recommended that I take for my subject of investigation "The Lateral Line System of the Toadfish (Bartrachus tau)." This I did with the consent of Dr. Ryder, who had worked on this subject. This was the first subject given out at the Marine Biological Laboratory and Allis' first publication on Amia was shown to me as a model for my work on Batrachus tau.

I can see Dr. Whitman sitting with us, showing us how to draw, telling us about the technique, making us feel that time was no consideration; our business was only to see and to get the results. The thoroughly scientific spirit which was evident and the complete absence of sensationalism was shown when he honestly told us that we should not waste our time with lectures. I was introduced to his ideas of original work or research, to his methods of work, to the idea that persistent and completely absorbed attention to one subject will lead to comprehension of much besides that. This was a new idea to me. Serial section cutting was new. It opened up a new aspect of work along biological lines. The atmosphere of that laboratory was an inspiration; the days were peaceful and quiet; there were on lectures nor anything else to distract attention from the work in hand.

Whitman was the editor of the *Journal of Morphology*, which was a new thing in the United States, and at that time the invitation to publish in it was one of the greatest honors to be had. I know particularly how I felt about that, for while I was working at Johns Hopkins University Professor Brooks said to me, "Conklin, I don't see how you will ever get this published." I got all the cold water that it was possible to throw on me, not only in that respect but in other respects too; but when I first came to Woods Hole I met Doctor Wilson, through whom I met Doctor Whitman. I was occupying the Johns Hopkins laboratory table at the Fish Commission, and Doctor Wilson came over to see me. To have met Wilson was one of the greatest events of my life. He told Whitman, who sent for me and asked me to come over to see him. I spent Sunday morning in his laboratory with him, the most inspiring morning I ever spent. He was most enthusiastic about my work and said, "I want you to promise me you will let me have this for publication in the *Journal of Morphology*." Well, I tell you that lifted a load, and it fired my enthusiasm. I am sure I might readily have gotten on the shelf and stayed there forever if it had not been for the stimulus and enthusiasm I received from Whitman and others who were here at that time.

I must not take more time in going over these earlier years. I had not intended to go into it as thoroughly as this, but I want to show you a few pictures from those early years and point out some of the people and some of the events con-

nected with them. [Eight lantern slides were shown.]

Let me rapidly run over some of the striking things which Whitman did. He had many helpers but he was the main stimulus. There was no landing-place, no boats, and no apparatus of any sort. They depended on the Fish Commission for all of these, but in the face of these conditions this is what Whitman wrote in his first report: "It is safe to say that the inside equipment will surpass anything hitherto provided for workers in seaside laboratories of this country." He gives his reasons for the necessity for the establishment of a marine biology station, ". . . the facts that (1) the ocean is the home of the lowest and oldest forms of life, (2) the great abundance and variety of marine life, (3) the fact that it has been relatively unexplored."

On July 17, Whitman gave his inaugural address. In it he said that he visioned this laboratory, and its goal, as "A national center for research in every department of biology"; that the laboratory should represent the whole of biology, both teaching and research, and should have the cooperation of scientific institutions. He always emphasized the breadth of the field that we ought to undertake to cover, and the freedom that should be allowed us in the development of the institution. He stood for the independence of the laboratory at a time when the Corporation and the Board of Trustees were almost unanimously in favor of turning the laboratory over to one of the Foundations. He insisted on the independence of the laboratory, and that it belonged to the biologists and should be governed by them.

I spoke of the customs of the Laboratory only last week to a visitor from a European laboratory. He asked me what the Laboratory's program was. I said there was none, that every man comes with his subject and does what he pleases with his work. That seemed a very amazing thing to him. "But the Director assigns the topic, doesn't he?" he said. No, the Director does not assign the topics. "Isn't it necessary for the Director to consult with the workers to see that they are not finding out things they should not find?" No, that is not necessary. "Don't you have to submit your publications to the Director to have them approved before they are printed?" No. "Well," he said, "this must be a heaven of freedom!" Compared to the things he had known, it was. Our characteristics differ from many of the European laboratories.

Whitman, as I say, was alone in giving advice on investigations during the first session of the Laboratory. He assigned to Miss Clapp, "The Lateral Line System of the Toadfish"; to Gardiner "The Anatomy and Embryology of Ascidians;" and to others he gave suggestions which are really quite up to date, for example "The Fecundation of the Egg of the Sea Urchin."

During the second session he had as assistants in research Dr. Ayres and Dr. Gardiner, and as assistants in teaching, Kingsley, McMurrich, and Humphreys. Whitman said of these men, as the Trustees said of Whitman, that they had served without any compensation, and had never thought of asking for any. He said of the assistants: "They served without compensation for the sake of the cause. An ideal that is more potent than self-interest, a conviction, a purpose, a hope that is strong enough to merge all personal ambition under its complete domination, proclaims a cause that need not blush to ask for financial aid." The spirit of the early years of the Laboratory was service, and this is the spirit of all great work. Whitman gave time, labor and money for twenty years without a cent of compensation. Lillie, his successor as Director for twenty years, has done the same, and all the founders and many early workers of these years of poverty did the same. All of our benefactors gave not for themselves but for the cause. In an age when the gesture that was assumed by that humorous organization, the "Veterans of Future Wars," was the begging hand, these workers assumed the gesture of the open hand. All great work is done only with the open hand, giving for the cause, a cause that is greater than personal preferment, the joy of creating and building which is the joy of giving. It is more blessed, more happy, to give than to receive, and it is more useful and more noble, more nearly immortal. The joy of life is progress, and the desire of scientists is to be immortal through their work. Blessed are they who have put their work into an immortal institution. For years and years it will continue to serve science and mankind. We may be forgotten, as many of our benefactors have been, and yet we can say with Huxley, "I am content to be remembered or not remembered so long as the truth is advanced."

On this occasion we remember with gratitude all who have put their thought and labor and money into the making of this great institution. We have named the roads in the Gansett Tract for those of the Penikese School, Agassiz, Whitman, Hyatt, Minot, Brooks and Clapp. We have tablets to some of these, and to Gardiner and Loeb in the foyer of this building. We have tablets and photographs of others in the reading room and stacks, and we have portraits, not merely to decorate these walls but to remind coming generations that they are indebted to those men and women who realized that it is more blessed to give than to receive, and that the only road to greatness is the road of service.

(On Tuesday evening, August 9, the seminar program of the Marine Biological Laboratory was replaced by exercises commemorating the fiftieth anniversary of the founding of the Marine Biological Laboratory. The addresses of Dr. Conklin and Dr. Lillie are reproduced here.)

THE BUILDERS OF THE MARINE BIOLOGICAL LABORATORY

Dr. Frank R. Lillie

President of the Corporation, Marine Biological Laboratory; Emeritus Professor of Embryology, University of Chicago

The development of the Marine Biological Laboratory has been the work of many minds and hands devoted to a common ideal of science and organization over a period of fifty years. Doctor Conklin has spoken of our early history and of the scientific leaders of that time. I would like to call to memory those who have contributed to the material upbuilding of the Laboratory, into whose inheritance we have entered. Some were scientific men, such as our Trustees, who contributed time and thought and devotion; others were laymen in science who sympathized with the spirit and aims of the Laboratory.

Among the former our first Director, Charles Otis Whitman, the living embodiment of our ideals of science and organization; Edward G. Gardiner of Boston, Clerk of the Corporation between 1890 and 1907, unselfishly and loyally serving the Laboratory, and shining in its times of crisis; and Gilman A. Drew, head of the invertebrate course, later of embryology, resident assistant director from 1910-1924; he was lame, but active, courageous, energetic and uncompromising, skilled in hand and brain, single in purpose; next to the architect he was the true designer of these buildings, over whose erection and furnishing he exercised the most vigilant supervision, so that their convenience is a monument to his memory.

Among the latter, the laymen, I would mention our treasurer, from 1899 to the time of his death in 1923, D. Blakely Hoar, a quiet, honorable gentleman of Boston, loyal to the Laboratory in all of its crises; and Charles D. Coolidge, also of Boston, member of our Board of Trustees from 1900 to 1921, a most distinguished architect, who rendered his services freely in the design and construction of our permanent buildings, and was also architect of the Woods Hole Oceanographic Institution and of the Bell Tower across the Eel Pond.

All whom I have mentioned have entered into their rest, and their works do follow them.

This Laboratory was opened in the summer of 1888 on a piece of land 78 x 120 feet, in a building of frame construction 63 x 28 feet which is still in use. It had no endowment.

Its development has been gradual, but not uniform. There have been periods of crisis, and periods of rapid development. Throughout it all the Laboratory has preserved its independent and unique organization. It sprang from Boston and Cambridge, but it has become national and international in its relations and influence. I like to think of this development as one example of the effects of the leaven of those great centers of liberty and learning.

Who are those who have contributed most by gifts of money to enable the Laboratory to render its services to the advancement of science?—not the scientific men themselves, for they could not, but others imbued with belief and faith.

In the first days Mr. Joseph S. Fay, and in still early days of the Laboratory, Mr. L. L. Nunn who helped to finance deficits; Dr. John C. Phillips who gave us our first harbor frontage. At the turn of the century the Carnegie Institution of Washington helped to balance the budget for three years. From then on Mr. Charles R. Crane balanced the budgets from 1904 to 1923. In 1913 Mr. Crane presented our first permanent building, the East wing of this building. In 1919 we entered into a campaign for enlargement and endowment. The National Research Council supported the campaign which lasted until 1924. The Rockefeller Foundation, Mr. John D. Rockefeller Jr., and the Carnegie Corporation contributed liberally on certain conditions which Mr. Crane met at great expense. The result put us on a high plateau of security which we still occupy. Then came forward that great president of the General Education Board, Wycliffe Rose, who secured money and endowment for the library and funds for erection of the dormitory and apartment house, and who also set in motion those forces that resulted in the establishment of the Woods Hole Oceanographic Institution.

But I have not yet done with Mr. Crane. His printed words are unfortunately few. But I take this from his remarks at the dedication of the Crane Building in 1914:

"I think we have come here particularly to celebrate the wonderful spirit that is back of the Woods Hole Biological Laboratory. It is very difficult to define that spirit, but I think we all know something of it and something is also known all through the scientific world. Without that spirit no amount of bricks and mortar and organization would be of any great service, but with that spirit the laboratory has been able to accomplish a very great deal with very simple means.

"For some time back it has seemed to be worth while to give this spirit a more substantial body. The spirit, as I see it, is very much like the spirit that President Wilson speaks so much of, the spirit of freedom and of cooperation, the fundamental spirit of democracy. In giving this spirit a more substantial body, we have been very fortunate in having with us Dr. Drew. I think we are all very happy at the wonderful result of his year's work. There is a rumor in circulation around here that Dr. Drew is a zoologist. I believe that rumor has

spread into the outside world, but I am very certain that we must all feel, after looking over the new laboratory, that Dr. Drew would have made his reputation as an engineer if he had a chance."

Another quotation is from his letter of resignation as President of the Corporation, August 8, 1925:

"Gentlemen: Twenty-two years have now elapsed since I became President of the Marine Biological Laboratory. I have enjoyed with you watching the growth of the Laboratory during that period. With the strong interest and support that is now assured, I feel that my own work has been completed and I hereby tender you my resignation which I ask you to accept.

"The future progress and prosperity of the Laboratory will always be a matter of great interest to me, quite as much as if I continued to be your President."

The reply of the Board of Trustees was as follows:

"Dear Mr. Crane: In regretfully accepting your resignation as the President of the Corporation of the Marine Biological Laboratory the Board of Trustees appointed the undersigned members of the Board a Committee to express to you their grateful appreciation of the invaluable services which you have rendered to the Laboratory. You have been a member of our Board since 1901 and President of the Corporation since 1905 and throughout this period you have been our constant supporter and friend. During your Presidency you have seen the Laboratory grow, from a relatively small beginning, to the largest and most complete biological laboratory in the world, with assets totaling more than two million dollars.

"Every year since your connection with our Board you have contributed most generously to the support of the Laboratory and for nearly twenty years you have lifted from our shoulders the burden of a large deficit. Only those who were members of the Board before your advent can fully appreciate the relief from financial worries and fears of suspension which your support has brought to us. Almost every year of your Presidency you have made notable additions to our estate, among which are the Kidder lot, cottage and annex, the Whitman and Ritter Cottages, and New Homestead and Mess Hall, the Bar Neck Property, the Gansett Property, our first permanent and fireproof laboratory which should be known as the Crane Building, and finally the completion of the fund for the building and equipment of the New Laboratory and the permanent endowment of your annual gift of twenty thousand dollars from the Friendship Fund.

"We are well aware that your interest in and support of the Laboratory has been a powerful factor in enlisting the cooperation of other benefactors and of great Foundations. If we have today one of the finest biological institutions in the world we owe it in large part to your faith and foresight.

"Best of all has been the spirit of the support of yourself and Mrs. Crane. Both of you have given generously and without any suggestion of conditions or interference. "The gift without the giver is bare", but you have given yourselves. Your spirit of helpful, friendly cooperation is the very spirit of this Laboratory which you have helped to create and confirm.

"We regret that you are to be our President no longer, but we rejoice to know that you will still be a member of our Board of Trustees and an ever cherished colleague and friend.

Sincerely and cordially yours,

E. G. CONKLIN,
T. H. MORGAN,
EDMUND B. WILSON,
Committee."

Today is our fiftieth anniversary, and two days ago was Mr. Crane's eightieth birthday. It is a fitting time to present to the Laboratory on behalf of the Trustees this portrait of Mr. Crane painted by his old friend, the Russian painter, Feodor Zakharov. After we are all gone these far seeing eyes and this friendly face in its familiar Woods Hole setting will long speak to our successors as the best friend the Laboratory has ever had.

THE CORPORATION MEETING OF THE MARINE BIOLOGICAL LABORATORY

(Continued from page 101)

State College of Washington; Samuel R. Magruder, Tufts Medical School; Daniel Mazia, University of Missouri; Edouard Albert Navez, Milton Academy; John Howard Northrop, Rockefeller Institute; John Stewart Rankin, Jr., Amherst College; Asa A. Schaeffer, Temple University; Eleanor H. Slifer, State University of Iowa; Paul Alfred Weiss, University of Chicago; Ralph Wichterman, Temple University.

The Corporation then elected, without contest, the entire slate prepared by the nominating Committee, as follows:

Treasurer, to serve for one year, Laurence Riggs, Jr.

Clerk, to serve for one year, Philip B. Armstrong.

Trustee emeritus, H. S. Jennings.

Trustees of the class of 1942: E. R. Clark, O. C. Glaser, R. G. Harrison, E. N. Harvey, M. H. Jacobs, F. R. Knowlton, Franz Schrader and B. H. Willier.

Memorials to trustees who died during the year were read to the Corporation rather than to the trustees, for it was felt that the members should hear of the men who for many years had served the Laboratory. The names of these trustees and of those who prepared the memorials are: W. M. Wheeler, memorial by G. H. Parker; M. J. Greenman, memorial by C. R. Stockard; E. P. Lyon, memorial by W. E. Garry; H. H. Donaldson, memorial by E. G. Conklin.

The assistant director, Dr. Packard, commented on matters relating to the current season. The attendance this year is as large as last and may exceed it slightly. Of special interest is the resignation of Mrs. Coombs who has worked for forty years in the mess, being in full charge of this important part of the Laboratory during the

last twenty years. The occasion of Mr. MacNaught's twenty-fifth anniversary of his coming to the M. B. L. as business manager was marked by the gift to him of a watch, presented by the many members of the laboratory who appreciate his tact, patience and devotion to the welfare of the institution. The Corporation voted to express their deep appreciation of the services of Mrs. Coombs and Mr. MacNaught.

The treasurer, Mr. Riggs, summarized the data already printed in the Annual Report. Financially the Laboratory is in good condition.

Mrs. Montgomery, the librarian, quoted extracts from the report of 1889 which showed that the need fifty years ago for complete files of all important journals in the principal languages was fully realized.

The technical manager, Dr. Pond, commented on the work done in his department, the highly varied and special types of service required by the investigators, the supply of electricity, and the work of the chemical room.

OBSERVATIONS ON SOME LOWER TURBELLARIA OF THE UNITED STATES

Dr. E. R. Jones

Associate Professor of Zoology, William and Mary College

There has been comparatively little work done on the morphology and taxonomy of the lower Turbellaria in this country. Among the earlier workers were Mark, Verrill, Woodworth, Leidy and Graff. Since Graff's visit in 1907 the only sustained work in this field has been that carried on by Kepner and his students at the University of Virginia, although recently Hyman, D. P. and H. M. Costello, and others have made contributions. For the past few years I have been collecting and studying representatives of the three lower orders of Turbellaria in various parts of Virginia and North Carolina as well as at Woods Hole. This work is still in its early stages and there are many forms which have not yet been positively identified. The present report will therefore deal with those forms previously unreported from this country which have been identified. Most of these belong to the order Rhabdocoela, although there is one representative of the Alleoecoela.

Perhaps it would be well before going on to say a few words about the Turbellaria in general. They are chiefly free living flatworms belonging to the phylum Platyhelminthes whose epidermis, excepting the majority of the Temnocephala which are frequently placed under this class, is entirely or at least in part ciliated and also bear rhabdites. The body lacks appendages and is filled with a more or less loose parenchyma which in the order Acoela becomes a central syncitium without sharp boundaries serving for digestive purposes. The other orders usually possess a simple or branched enteron which, with the exception of a few forms, is without an anus. All forms with the exception of a marine triclad are hermaphroditic. Development is usually direct with larval stage present in only one Rhabdocoele and many Polyclads. There are six orders usually recognized—Acoela, Rhabdocoela, Alloeocoela, Tricladida, Polycladida, and Temnocephala. Of these the first four are very closely related and it is probable that they will shortly be included in one order. In recent classifications by German workers the Tricladida have already been included in the order Alloeocoela.

The present study has been confined to the three lower orders. Of these the Acoela lack an enteron, the Rhabdocoela have a rod or sack-shaped enteron and the testes are usually compact, while the Alloecoela have an enteron which may be sack-shaped or may bear lateral diverticula, with the testes usually follicular. The worms are small in all of these orders, and in most species do not exceed a few millimeters in length.

In some collections made from the brackish water of Currituck Sound at Currituck, North Carolina, in the summer of 1933, a number of specimens of a worm belonging to the species *Macrostomum* were encountered. These proved different from the previously described species of this genus and consequently have been described as a new species with the specific name *stylopencillum*. This name was considered appropriate because of the sharp, straight, needle-like stillet associated with the male copulatory organ. A detailed description of this species is included in a monograph on the genus *Macrostomum*, by Dr. Frederick F. Ferguson of the University of Virginia, which will be published in the near future.

Omalostomum schultzei, another representative of the family Macrostomidae, has been found in considerable numbers at Woods Hole during the past three summers. This is apparently the first record of its occurrence anywhere since Claparède in 1863 obtained his specimens from the coast of Belgium and described them as a new species. Specimens obtained at Woods Hole are slightly larger than those found by Claparède but otherwise are similar. No representatives of the family Solenopharyngidae have hitherto been reported from this country. *Solenopharynx flavidus* described by Graff in 1882 has been reported from Naples, Trieste and various other places in Europe and several specimens have been obtained at Woods Hole and around Norfolk, Virginia. *Sopharynx oculatus* on the other hand is much less common. It was described from the Black Sea by Pereyaslawa in 1892, has been seldom reported in the literature, and only one specimen has been found at Woods Hole. No others have been found in this country. Under the family Koinocystidae,

Polycystis mamertina described by Graff in 1874, is widely distributed in Europe and occurs abundantly at Woods Hole.

In the family Cylindrostomidae under the order Alleoecoela, Beauchamp in 1913 described a new species which he called *Monoophorum graffi*. This he obtained from pools of brackish water on the coast of southern France. A number of specimens which evidently belonged to this species were obtained in the collections from Currituck, North Carolina, which have been previously mentioned. Since Beauchamp's original description is the only previous record of the occurrence of this species and since none of his specimens were sexually mature there remained some doubt as to its actual systematic position. As a result of the study of sexually mature American representatives of the species it was found that the female reproductive system includes among its accessory structures, a vagina opening directly to the exterior for the reception of sperm cells, a bursa seminalis for the storage of sperms until the eggs reach maturity, and an unpaired ductus spermaticus for the passage of the sperm cells from the bursa into the ovary where fertilization occurs. The presence of these accessory structures necessitates the removal of this species from the genus *Monoophorum* and its inclusion in the genus *Enterostomula*.

The excretory system of this species also proved of interest because of its unique construction. It consists of a pair of tubules lying one on either side of the mid-ventral line and extending from the region of the cerebral ganglia to the base of the penis. They fuse together at their posterior ends to form a urinary sinus or vesicle which opens into the penis sheath into which the liquid wastes are presumably discharged. In cross section it is seen that on its ventral side each tubule is lined with a chitin like material arranged in a peculiar spiral formation. Radiating from this are a number of vacuolated spaces so that the tube seems to be a compound structure composed of a number of smaller tubules. This type of excretory system seems to be peculiar to this species.

(This article is based on a seminar report given at the Marine Biological Laboratory on August 2.)

REGENERATION OF NITROGEN IN THE SEA

Dr. Charles E. Renn
Instructor in Biology, Harvard University

Nitrogen exists in the sea in a variety of forms —as gaseous nitrogen; as ammonia, nitrite, and nitrate; as soluble organic nitrogen of varying degrees of stability; and as a constituent of living and dead animals and plants. The prevailing crop of living things in the sea is limited by the rate at which nitrogen in some available form can be supplied to the superficial, illuminated waters. This rate, in turn, is set by the velocity of several interrelated bacterial processes. With the possible exception of small quantities of available organic nitrogen produced by animals, those forms of nitrogen which may be synthesized into new plant cells are produced by bacterial activity.

Conditions of life in the sea are somewhat different from those prevailing in soils and in fresh water where the nature of the bacterial processes involved in nitrogen regeneration have been very adequately demonstrated. Nevertheless, we have always felt that the processes in all three cases were identical. The various special groups of bacteria involved in the nitrogen cycle of soils, sewage, and fresh water have been isolated from sea water and from marine muds. Certain of these bacteria, autotrophic, nitrifying organisms, have been isolated from muds only. The scheme demanded by chemical data requires that the conversion of nitrite to nitrate take place in the upper waters, in the zone of production. It seems unlikely that this essential combination can otherwise reach the superficial layers with sufficient rapidity to maintain the standing crop.

In experiments at the Woods Hole Oceanographic Institution, Rakestraw, von Brand, and Renn studied the decomposition of pure strains of diatoms and their subsequent regeneration. When suspensions of diatoms comparable to those occuring in the sea are placed in the dark the cells rapidly break down under bacterial attack and considerable bacterial cell substance is produced during the first two weeks. Ammonia is rapidly liberated in quantities equivalent to the original nitrogen content of the diatoms and after a period of six weeks to two months this is oxidized to nitrite. Following the development of a nitrite maximum the third step begins and nitrate appears. The whole decomposition phase requires from four to six months. When the system is re-illuminated and seeded with a small quantity of diatom material the nitrate is quantitatively converted to living diatom material. These cycles have been repeated several times and in no case have unavailable nitrogenous materials appeared in measurable quantities.

These experiments satisfy us that the sequence of nitrogen regeneration in the sea is essentially the same as that in soils and fresh water. The rates at which the processes take place are such as to maintain the standing crop of plankton without drawing on such other sources as atmospheric nitrogen (through fixation), stable dissolved organic nitrogen, etc.

(This article is based upon a seminar given at the Woods Hole Oceanographic Institution on July 21.)

REPORT ON THE CURRENT TRIP OF THE "ATLANTIS"

Dr. H. C. Stetson
Research Associate in Paleontology, Harvard University

As part of the geological program which is being carried out by the Woods Hole Oceanographic Institution the *Atlantis* recently made a trip south along the continental slope to the latitude of Cape Charles for the purpose of obtaining long cores at significant points.

This work was made possible by some new charts of the off-shore bottoms which have recently been constructed by Dr. A. C. Veatch, working under the auspices of the Geological Society of America, from new data furnished by the U. S. Coast and Geodetic Survey. The submarine canyons cutting the continental margin have been well known for some years, but the areas in between were marked by only comparatively few soundings. Recently it was decided to apply the same detailed charting methods to the whole continental slope from the latitude of the Chesapeake to Georges Bank, and at present only small portions remain incompleted. The U. S. Coast and Geodetic Survey in most cases used the same technique, called radio acoustic ranging, which had been developed in charting the canyons. Briefly, this method consists of using anchoring buoys which carry a radio receiving and sending set, while the surveying vessel steams on her course, using her echo sounding apparatus continuously. At intervals a bomb is dropped overboard and the sound waves, traveling through the water, are picked up and radioed back to the ship. Thus both position and distance from the buoys is known.

The new charts show that the continental slope instead of being the relatively smooth descent to the ocean basin that it had formerly appeared, has been deeply channeled, and the canyons, that had once seemed so striking, are but parts of a much larger whole. It is a maturely dissected terrain of well defined valleys with intervening divides reaching 8000 feet and more below present sea level. By means of accurately located cores it is hoped that something can be learned about the sedimentary formations in which these valleys have been cut, and information obtained about the time of cutting. The problem is of course connected with the origin of the submarine canyons, a topic that has aroused so much controversy in recent years. It cannot yet be definitely proved whether such a vast amount of erosion took place under the air or under the water.

The apparatus used in obtaining the cores was the tube invented by Dr. Charles S. Piggot of the Geophysical Laboratory of the Carnegie Institution. The bit is driven into the sediment by a cartridge which is fired on contact with the bottom. This instrument will take a ten foot core which is about twice as long as can be obtained by any other type of coring tube. It furnishes the geologist with a means of penetrating the mantle of recent silt and getting at the older formations which lie below. In addition a ten foot core obtained far off shore where the rate of sedimentation is slow may represent an appreciable portion of geologic time.

Submarine geology is necessarily limited in its scope, but with improved methods of sampling, with the adaption of seismic prospecting methods to marine conditions, and with more accurate and detailed charts much can be learned about the submerged portions of the continents.

THE ANNUAL CONCERT OF THE WOODS HOLE CHORAL CLUB

The Woods Hole Choral Club will hold its twelfth annual concert at 8:30 on Wednesday evening in the Woods Hole Towne Hall. It will present a carefully selected program which will be of interest to music lovers of Woods Hole.

The first part of the program consists of sacred music, and is made up of works by Russian, German, and Dutch composers. Lvovsky's "Gospodi Pomiluy," which has been so popular at presentations of the Choral Club in the past, will be given again this year. The second half of the concert consists of music in a lighter vein, and includes folk-songs and various other compositions, of varying moods and nationalities. Margery and Edith Mitchell, daughters of Dr. Phillip Mitchell of Brown University, will sing a solo duet during one of the numbers in this part of the program.

Tickets may be obtained from members of the Club for twenty-five and fifty cents; they will also be available at the door.

The program which has been selected for the concert is as follows:

Prayer of Thanksgiving — *Netherlands Folk-Song*
Only Begotten Son — *A. Gretchaninoff*
Of Thy Mystical Supper — *A. F. Lvoff*
Gospodi Pomiluy — *Lvovsky*
Then Round About the Starry Throne — *Handel*

(Intermission)

Farmer, What's That in your Bag? — *O. di Lasso*
Chanson (La Nuit Froide et Sombre) — *O. di Lasso*
Gypsy Song — *Brahms*
Tiritomba — *Italian Folk-Song*
Cuckoo Song — *Lorenz Lemlin*
Soloists: Margery and Edith Mitchell
The Old Woman and the Pedlar — *English Air*
Dusk of Night — *A. Arkhangelsky*
Psalm 150 — *Cesar Franck*

—*B. I. G.*

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris Gorokhoff and Hazel Goodale.

Entered as second-class matter, July 11, 1935, at the U. S. Post Office at Woods Hole, Massachusetts, under the Act of March 3, 1879, and re-entered July 23, 1938.

Introducing

Michael J. D. White, Lecturer in Zoology, University College, London; Rockefeller Foundation fellow at Columbia University and the Marine Biological Laboratory.

Dr. White's laboratory here in Woods Hole tells the story of his scientific interests and research. Many of the photographs of chromosomes which he has taken himself line the walls, cases of mounted insects are on the shelves and cages of living grasshoppers on the floor. The work which is being done here centers on chromosomes; Dr. White is an experimental nuclear cytologist. His most important study has been on chiasmata and crossing-over, effects of X-rays on mitosis and meiosis, spiral structure of chromosomes, and heteropycnosis of sex-chromosomes.

This research was begun in London, where the teaching of morphology requires so much of his time that Dr. White appreciates a year's leave of absence to come here to devote his full attention to investigation. He spent this winter at Columbia University and went to Mexico this spring to collect material for work on anomalous types of meiosis. In return for lectures given there, Dr. White was honored with a parchment diploma naming him, "Professor extraordinario."

Traveling is not a new experience for Dr. White, for although he was born in London, he was brought up in Italy and France, living in each place six and eight years respectively. He speaks three or four languages and feels at home in any country. He was educated at University College, London, receiving his B.Sc. degree in Zoology and Physiology, and an M.Sc. a year later. Before occupying his present position as Lecturer in Zoology, Dr. White was assistant lecturer at University College, London.

Publications by Michael White include about ten papers on his research work and a recently published book, *The Chromosomes*. At the September meeting of the Genetics Society here, he will give a paper on studies he has made on material from Haiti.

Tennis and swimming do not appeal particularly to this Englishman; he gets his exercise chasing bugs all over the Cape. He has done a lot of mountain climbing, but in this flat part of the country has to substitute duck shooting—tin ones—at Oak Bluffs. Dr. White says he likes America very much and has enjoyed his summer at Woods Hole. He sails for England on September 6th on the *Ile de France*. —*M. F. M.*

Presentation of Portraits of Professors Conklin and McClure to Princeton University

Princeton University has many portraits of Trustees and Presidents, but, except for Dr. Fine, professor of mathematics, in whose memory the Institute for Advanced Studies was founded, none of their illustrious professors has been so honored until this year. On Sunday, June 19, during the Commencement celebrations, the University was presented with portraits of Professors McClure and Conklin, who both retired five years ago. The occasion was the fiftieth anniversary of Professor McClure's graduation and his portrait was presented by his classmates with a speech by their representative, Dr. Livingston Farrand, President of Cornell until this year. Professor Conklin, who, besides being noted for his work on the development of Amphioxus and Crepidula, is one of the M. B. L. trustees and well known at Woods Hole, owed his portrait to the gratitude of his former students, from among whom Dr. George Berry, professor of bacteriology at the University of Rochester, was selected to present the portrait. Dr. Dodds, President of Princeton University, formally accepted them both.

The portraits are the work of John Young-Hunter, son of the famous Edinburgh portrait painter. Those who have seen the portraits say that Professor McClure's is a "speaking likeness", whereas Professor Conklin's is a "listening likeness", his students saying that he looked like that when they came into his room to tell him their troubles. —*A. M. M.*

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
August 14	6:56	7:20
August 15	7:38	8:04
August 16	8:24	8:48
August 17	9:10	9:39
August 18	9:58	10:36
August 19	10:57	11:38
August 20	11:56	
August 21	12:41	12:59
August 22	1:40	1:56

In each case the current changes approximately six hours later and runs from the Sound to the Bay.

ITEMS OF INTEREST

At the annual meeting of the Woods Hole Oceanographic Institution Dr. A. E. Parr of Yale University was elected to the Board of Trustees, replacing Dr. Benjamin M. Duggar. Professor A. F. Spillhaus of New York University was appointed investigator in physical oceanography for a period of one year.

At the *Staff Meeting* of the Woods Hole Oceanographic Institution on August 11, Drs. G. H. Parker, A. Abramowitz and C. M. Osborn presented a paper on "Fish and Internal Secretion."

DR. W. F. HAHNERT, formerly assistant professor of zoology at Ohio Wesleyan, has been appointed to position of associate professor there. He is a junior instructor in the invertebrate course of the Marine Biological Laboratory.

DR. ROLAND E. MILLER has been appointed assistant professor of anatomy at Wake Forest Medical School in North Carolina. He took his Ph.D. degree in zoology this June at the University of Missouri.

DR. R. P. HALL, associate professor of biology at University College, New York University, has been appointed to a full professorship of biology.

Among the biologists who have come recently to Woods Hole are: Professors C. E. McClung, W. C. Curtis, L. L. Woodruff and J. E. Kindred.

DR. EDMOND J. FARRIS, fellow in anatomy and in charge of operations at the Wistar Institute of Anatomy and Biology visited Woods Hole for several days this week. Dr. Farris was accompanied by Dr. Richard McCoy, in charge of nutrition research in the Department of Biochemistry of the Institute.

DR. J. F. NONIDEZ, professor of anatomy at Cornell University Medical College, who left in the middle of July for a trip to Cuba, has returned to Woods Hole.

PROFESSOR and MRS. ROBERT CHAMBERS left on Wednesday by car for East Jaffrey, New Hampshire, where they will spend a few days before returning to Woods Hole. Their son, William, accompanied them.

In the sailing races this week the hand of Preston Copeland was caught between his boat and another. The accident necessitated the amputation of one of his fingers.

Mr. Wigfall, the father-in-law of Professor Walter S. Root of Syracuse University, died suddenly this week. Mr. and Mrs. Wigfall have been ocupying Professor Root's cottage while he is abroad with his wife.

MR. and MRS. ARTHUR RUGH visited their son Dr. Roberts Rugh at Woods Hole on Sunday, Monday and Tuesday. Mr. Rugh teaches at Yenching University at Peking, China, and at the Peking University Medical College. At a gathering at their son's house Mr. Rugh related his experiences in war-torn China including the time when Ambassador Johnson placed him in charge of evacuating twenty-five American men, women and children from the war zone. After great difficulty he got them on a boat from Nanking to Hangchow which carried 1650 people although its regulation capacity was only 500.

Final Scores of the Tennis Tournament

The playing on Friday afternoon of the final matches of the Men's and Junior Singles marked the completion of the 1938 Tournaments of the M. B. L. Tennis Club. On Wednesday in the semi-finals Hekhuis defeated Frank 6-3, 7-5 and Miller defeated Rugh 6-1, 6-2. In the final round Miller was victorious over Hekhuis by a score of 6-1, 6-2. The Junior finals brought Ted Jones and Joe Crossley together with Jones the winner by a score of 6-4, 6-2. After the matches Miller received the Tennis Club singles cup and also a Blakiston individual trophy and Patten and Miller were given Blakiston trophies for the men's doubles. Mrs. Saunders presented the Saunders Junior trophy to Ted Jones.

The M. B. L. Club Ping Pong Tournament

Of the twelve first round matches scheduled in the M. B. L. ping pong tourney seven have been completed. The great interest displayed by the numerous spectators has been well rewarded in that, for first round battles, all matches have been remarkably closely contested.

In the men's singles Coker eliminated Levin, 21-17, 16-21, 21-18, in a battle marked by considerable steadiness of play. Chambers advanced to the second round by defeating young Ludwig by the close margin of 18-21, 21-18, 21-19. The Timlin-Hutchins match was decided by two deuce games, the latter player emerging the winner by the score of 22-20 22-20. Harvey, M. B. L. ping pong champion of 1935, was defeated by Kriete in a contest of hard driving, 21-19, 22-20. Thompson was winner over Silber in a three-game match, 21-17, 17-21, 21-12.

In the women's singles Bernstein eliminated M. Mast, 21-17, 21-10, while Guttman was forced to go three games, 21-19, 19-21, 21-16, in order to advance to the second round over E. Mast.

The remaining first-round matches are to be completed by Tuesday, August 16. —*L. Levin*

THE M. B. L. TENNIS CLUB AND ITS TOURNAMENTS

During the past week three of the Tennis Club Tournaments have been completed. On August 4th, Patten and Miller defeated Buck and Duryee by the scores 6-1, 6-3 to win the championship. The match was featured by some very fine net play by the winners.

Individual trophies, donated for the first time this year by P. Blakiston and Sons, are to be awarded the winners of the men's doubles and singles after the completion of the latter on August 11th.

On August 6th Mrs. Burton won a hard-fought match from Sally Elwyn after losing the first set 4-6, 6-4, 6-3. Both players were in top form and the match was a fitting conclusion to what was perhaps the best woman's tournament that the tennis club has seen. Both finalists lost the first set of their semifinal matches. In these matches Mrs. Burton defeated Miss Musser 3-6, 6-4, 6-2 and Miss Elywyn defeated Miss Melland 4-6, 6-3, 6-2.

August 7th and 8th saw the semifinals and finals of the mixed doubles tournament. In the first semifinal match, Sally Elwyn and Spinnler won a berth in the finals by defeating Miss Melland and Buck, 7-5, 6-1. In the other match Mrs. Norman and Harlow won handily from Betty and David Elwyn 6-1, 6-3. In the final match Mrs. Norman and Harlow outsteadied their opponents in two close sets, 10-8, 7-5.

The men's singles tournament has reached the semifinal round as this goes to press. In the upper bracket Frank and Hekhuis won their quarter final matches by default. In the lower bracket Rugh defeated Ryan 8-10, 6-4, 6-4 while Miller bested Spinnler 6-3, 6-1. Rugh's match was interrupted by rain with the score 5-4 in the third set and was finished Wednesday morning.

In the finals of the Junior tournament to be played August 12th Ted Jones will meet Joe Crossley. This match should bring out some excellent junior tennis since neither player has lost a set thus far. Indeed Ted Jones won his semifinal match without losing a game.

INVERTEBRATE CLASS NOTES

"Hi, angel! Hi, yourself, Noah!" These were the greetings heard at the beginning of the Invertebrates' first field trip on August 4. The four eager teams sailing on the *Winifred* left the dock shortly after the scheduled time, waving goodbye to their compatriots in the *Nereis*. For a while the latter seemed fated to study the anatomy and physiology of a balky engine rather than the ecology of the invertebrate specimens frequenting a sandy shore. After successful wielding of gaskets and monkey wrenches, gasoline ceased to spurt from the cylinder head and the entourage was off to a flying start.

The goal of Lackey's Bay was attained and the example of the four early bird teams of the *Winifred*, who were wading around in the water with sea-buckets, nets and shovels, was followed. After a time, what seemed the most insurmountable difficulties of a collecting trip were conquered. We became accustomed to reeling off complicated zoological terms with nary a flicker of an eyelash. Crystallizing dishes were put to their proper use rather than being used to determine the direction of currents and tides. Barnacles were found by the method of searching and not the one of falling off of a high rock into them. Incidental knowledge gleaned from the trip was that water can be very wet and uncomfortable, that seaweed and algae makes anything but a good footing, and that the instructor knows far more than a poor invertebrate student can ever hope to.

The experience of a field trip was repeated the following days with a few minor omissions and additions at Stoney Beach.

A Major League baseball fan would have had his education completed and enhanced had he seen the soft ball game played between teams from the South and North side of the Invertebrate laboratory on Saturday afternoon. While the traffic from base to base is usually one way, he would have seen one undaunted member of the South team run like a shot out of a gun from third back to first. He would have seen a first baseman, who was momentarily deranged when a North side batter finally hit the ball, run over to second and almost get tagged out by a member of his own team. He would have seen an umpire with no subtlety at all about his partiality. Dr. Matthews had an agreement with the scorekeeper whereby she would let him know when the score was even and he could let his decisions lean the other way. When the score had stayed at 12-8 in favor of the South side for what seemed about half of the game, the unanimous decision was reached that swimming was a much better form of recreation anyway and the extra-curricular session of the class was adjourned.

It is assumed—we know not how correctly—that we know all about Coelenterata, since we have finished four days' intensive study of the little diploblasts under Dr. Crowell's tutelage. We are at present pursuing exceedingly active Platyhelminthes around a slide with out disappointingly stationary microscope, and endeavoring to identify the minute structures which Dr. Hadley assures us are really there.—*E. L. Jordan.*

STAFF OF THE MARINE INVERTEBRATE COURSE OF THE MARINE BIOLOGICAL LABORATORY

Back Row: Drs. T. H. Bissonnette, J. C. Wightman, F. R. Killie, W. F. Hahnert. **Second Row:** Drs. A. M. Lucas, P. S. Crowell, Jr., S. A. Matthews. **Front Row:** Drs. A. J. Waterman, C. E. Hadley, J. S. Rankin.

THE COLLECTING NET

Vol. XIII, No. 6 SATURDAY, AUGUST 20, 1938 Annual Subscription, $1.50 Single Copies, 30 Cents.

STUDIES OF THE FACTORS INFLUENCING REGENERATION

DR. L. G. BARTH

Assistant Professor of Zoology, Columbia University

Regeneration is really a special case of the general problem of differentiation. It has long been recognized by embryologists that the parts of an organism are not irreversibly determined. A highly differentiated structure or even a whole organism may dedifferentiate and redifferentiate into a new structure or organism. Forty years ago Hans Driesch, after finding that several marine eggs behaved as if the parts were equipotential, turned to the adult and, using the ascidian Clavellina, showed that even such a highly organized individual might form an equipotential system in which any part of the adult might reconstitute an entire animal. Morgan likewise recognized that the problems of development and those of regeneration were closely allied and used Tubularia to show that polarity was not irreversibly determined but could be literally reversed. Child for many years (*Continued on page* 129)

M. B. L. Calendar

TUESDAY, August 23, 8:00 P. M.

Seminar: Dr. E. Eleanor Carothers: "Cytological effects of X-rays on grasshopper embryos."

Dr. T. N. White: "Recovery of Arbacia eggs from the effects of high intensity X-rays."

Dr. P. S. Henshaw: "The effect of X-rays on Arbacia sperm."

Dr. J. Furth: "Quantitative studies on the effect of X-rays on mammalian cells and on the mode of X-ray action."

FRIDAY, August 26, 8:00 P. M.

Lecture: Dr. Peter Gray: "Possibility of affecting developmental pattern by electrical means."

STRUCTURAL AND KINETIC ASPECTS OF CELL DIVISION

DR. ROBERT CHAMBERS

Research Professor of Zoology, New York University

In a recent publication, Schechtman has offered pertinent data for the conception that cell division is related to a sol-gel transformation of cytoplasm involving the inward growth of cortical material at the equator of the cell. The evidence he presents is based on the behavior of the vitally-stained egg cortex of the Pacific Coast newt, *Triturus torosus*. The contraction and later expansion of the cortex in the furrow, during its advance, is shown by the streaky lines of the stained cortex. At about the same time a publication appeared by Dan, Yanagita and Sugiyama with similar evidence from studies on the dividing eggs of a Japanese sea-urchin, *Mespilia globulus*. Dan and his co-workers observed the movements of the surface of the egg during its cleavage by the successive positions of kaolin particles adhering to the surface of the egg.

Chalkley, followed by Schechtman, has sug-

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CLASS IN INVERTEBRATE ZOOLOGY OF THE MARINE BIOLOGICAL LABORATORY

Back row (standing): R. Sperry, C. Hamann, D. Rollason, J. Snedecor, Mary Pierson, W. Belda, Lucena Jaeger, Mathilda Schneider, Nellie Harris, Elsie Taber, Eleanor Sheehan, Helen Ward, L. Fraser, D. de Lisa, W. Haines, R. Griffiths, S. Joseph, P. Morrison, B. McDonald, Josefina Acosta, d'A. Welch, F. Ferguson, T. Ryan. **Middle row (standing):** R. Fleming, T. Sackett, R. Smith, B. Schaeffer, Lydia Hall, Carolyn Trowbridge, Evelyn Nadler, Mary Sanders, Mary Hoagland, Charlotte Root, Marilyn Cooney, Lorna Wells, Harriet Towle, Helen Fahl. **Front row (standing):** M. Dobbelaar, J. Davis, H. Brown, R. Alexander, R. Reyer. Staff: J. Wightman, Dr. S. Crowell, Dr. J. Rankin, Dr. C. E. Hadley, Dr. T. H. Bissonnette, Dr. S. Matthews, Dr. F. Kille, Dr. W. Hahnert, Dr. A. Waterman. **Front row (sitting):** Margaret Kellogg, Margaret Deringer, Frances Bigler, Todd Crane, Sylvia Kerrigan, Genevieve Love, Edith Williams, Shirley Gale, Elizabeth Jordan, Irene Graves.

gested a parallel between the protrusion of a pseudopodium by an amoeba and the intrusion of the cell-surface during cleavage, both being considered a localized growth of peripheral cytoplasm at the expense of the more fluid elements of the cell. This parallel was also suggested when I presented a motion-picture film of segmenting sea-urchin eggs at the Congress of Experimental Cytology in Cambridge, England, in the summer of 1933.

Reversible gelation is a well recognized property of protoplasm. A peculiar feature is that the gelation tends to involve only restricted regions of the protoplasm. An example of this is the pseudopodium in some of the fresh-water amoebas in which a reversible granulo-gel surrounds a column of a flowing granulo-sol. Another is the gel in Echinoderm eggs of the progressively enlarging sperm-aster, the periphery of which is surrounded by a flowing cytoplasm in the sol state. Still another example is the gelated, granular cortex beneath the protoplasmic surface of certain cells.

The granular cortex of the Echinoderm egg is indistinguishable to the eye from the underlying flowing cytoplasm but its presence can be detected with the microneedle. In the fertilized egg it attains more appreciable proportions and is readily visible over the hyaline zone of centrifuged Arbacia eggs by the pigment-granules which remain imbedded in it.

On the external surface of the gelated cortex is the protoplasmic surface-layer, which preserves the integrity of the underlying protoplasm. It is still a question whether the protoplasmic surface-layer is a flowing film or is an integral part of the gelated cortex.

When a cell is about to undergo division a difference develops between the surface at the poles and at the equator. An illustration of this occurs in tissue-culture in which blebs or hyaline protrusions appear first at the polar tips of dividing cells. Subsequently, the blebs extend over the rest of the cell when the division has been about completed.

In Echinoderm eggs the stability of the cleaving furrow maintains the original plane of cleavage even when a dividing egg is cut through with a microneedle diagonally to the cleavage plane. The continuation of the cleavage then detaches from each cut portion that part which ordinarily belonged to the other blastomere.

The semi-rigid nature of the wall of the cleavage furrow is strikingly demonstrated by tearing the polar surface of a segmenting Arbacia egg after all extraneous coats have been removed (by shaking and KCl treatment), the operation of tearing being done while the eggs are in a solution of KCl isosmotic with sea-water. The absence of divalent cations in the medium permits the continued outflow of the granular interior of the egg through the tear. The ensuing disintegration of the torn, incipient blastomere has no effect on the stability of the walls of the furrow which continues to advance even while the interior of the other forming blastomere escapes through the open connection between the two blastomeres.

The thickness of the advancing wall of the furrow was determined by introducing a drop of paraffin oil into the center of the equator of a dividing Lytechinus egg. The oil-drop, at first spherical, became indented when the external surface of the advancing furrow was 4 to 5 micra distant from the surface of the oil. This distance was maintained while the oil-drop became constricted into two portions connected with a slender thread of oil. The dividing of the oil-drop into two spheres represents work being done by the advancing furrow.

The experiment of introducing an oil-drop into the equator of a dividing egg was repeated this summer in order to obtain a photographic record. In the several experiments done with the Arbacia egg (available at Woods Hole), the oil, instead of being pinched in two, was displaced by the advancing furrow into one or the other blastomere. This difference in behavior between the Lytechinus and Arbacia egg may be related to the different size-relations of the two eggs. The Lytechinus egg averages 110 micra in diameter and the introduced oil-drops about 40 to 60 micra. The Arbacia egg is much smaller and the injected oil-drops averaged only 10 to 20 micra in diameter.

The mutual reversibility of the sol and gel state in protoplasm is generally recognized. Mechanical agitation with microneedles brings about a reversal of the gel to the sol state of the aster and of the cortex of Echinoderm eggs. The subjection to high hydrostatic compression was found by Brown to cause the sol reversal of the cortex of the fertilized Arbacia egg. A direct relation of gel-sol reversal to maintenance of the cleavage furrow has been demonstrated by applying either method to the Arbacia egg during its cleavage. With the reversal to the sol of the cortex particu-

THE COLLECTING NET was entered as second-class matter July 11, 1935, at the Post Office at Woods Hole, Mass., under the Act of March 3, 1879, and was re-entered on July 23, 1938. It is devoted to the scientific work at marine biological laboratories. It is published weekly for eight weeks between July 1 and September 15 from Woods Hole, and is printed at The Darwin Press, New Bedford, Mass. Its editorial offices are situated on Main Street, Woods Hole, Mass. Between June 1 and October 1 communications should be addressed to Woods Hole, Mass.; at other times they should be directed to THE COLLECTING NET, Garrison, N. Y. Single copies, 30c; subscription, $1.50.

larly in the wall of the furrow, the furrow disappears and cleavage is stopped.

It is of interest to note that the reversal may be not only temporary but localized to the immediate region which is agitated. For example, if the wall of the furrow is punctured with a microneedle and the needle thrust back and forth repeatedly, the furrow about the punctured spot flattens out without affecting the advance of the furrow along the remainder of the equator.

A comparison of the influence of the aster and of the cortex on cleavage shows that the time of sol reversal of the one or the other is of prime importance. For example, during the early amphiaster stage a sol reversal of one or both asters has a decided effect on the orderly sequence of events while a sol reversal of the cortex, by tearing it and drawing out strands of protoplasm with a needle, has none. On the other hand, when the cleavage furrow is well under way, a reversal of the asters to the sol state has no effect, while a similar reversal of the cortex at the furrow immediately stops cleavage.

Three superficial regions of the Arbacia egg can be distinguished which exhibit movements during cleavage: one, an extraneous protein-like coat; two, a cortical semi-rigid zone of cytoplasm underlying the protoplasmic surface-layer; and three, a sub-cortical, fluid cytoplasm separating the cortex from the gelated asters. The sub-cortical cytoplasm exhibits a definite vortical flow sweeping around the asters from the polar regions of the egg toward the wall of the cleaving furrow at the equator. The corresponding movements of the gelated cortex and of the overlying extraneous material have been described by Dan as being of the nature of an expansion at the poles accompanied by a contraction at the equator. These surface movements may be due to a dragging effect induced by the vortical currents in the sub-cortical cytoplasm.

The existence of properly directed vortical currents in the sub-cortical regions is essential for the advance of the gelating wall of the cleavage furrow. Stretching a dividing egg in the direction of its long axis does not impede the vortical currents, and cleavage takes place. On the other hand, stretching the egg along its equatorial axis upsets the currents and stops cleavage.

Since the advancing wall of the furrow is a gel, it is suggested that the advance is effected by the reversal to the gel state of fluid cytoplasm which is being carried to the equator by the sub-cortical, vortical currents.

There is evidence for a causal relation between the initiation of streaming in the cytoplasm at or near the surface of a cell and the approach of the nucleus to that surface. This has been shown in the fresh-water amoeba in which moving the nucleus within the amoeba to any spot on its surface induces the formation, at that spot, of a streaming pseudopodium. In the Echinoderm egg the so-called resting nucleus has no such effect. The assumption is, therefore, that the currents of flow are induced by the nucleus only during its telophase. It is at this stage that the elongation of the mitotic spindle, described by Belar, causes the approach of the two daughter nuclei to the polar surfaces of the egg.

Concerning the egg nucleus there is an early observation of shifting the female pronucleus of the Cerebratulus egg, with a microneedle, to a new position beneath the surface of the egg after the formation of the first polar body. The formation of the telophase nucleus of the second maturation spindle was accompanied by the appearance of vortical currents about the polar nucleus followed by a localized elevation of the egg's surface to constitute the second polar body.

The existence of a rhythm in the successive cleavage periods of the egg is a significant feature. The cleavage furrow after being experimentally suppressed, by agitation with a needle or by hydrostatic compression, will quickly reform if the suppressing agent is removed during the earlier part of the cleavage stage.

If the suppressing agent is not removed until a later state, or is applied late and then removed, the furrow does not reform and the egg returns to a spherical shape. During this time the spindle between the two telophase nuclei disappears. Both nuclei then become less excentrically located and the sub-cortical currents cease. When the time for the second cleavage arrives two spindles appear and the consequent succession of events results in a simultaneous cleavage of the egg into four blastomeres.

In summary, the various factors apparent in the division of the cell are as follows: In the Echinoderm egg the elongation of the spindle during anaphase and telophase brings the daughter nuclei into excentric positions with respect to the surface of the egg which up to this time has been spherical. The approach of the nuclei to the two opposite surfaces of the egg initiates streaming movements in the cytoplasm. The opposite poles then bulge resulting in an elongation of the egg. The sub-cortical vortical currents sweep around the two asters and add gelating material to the inwardly growing cortex of the walls of the cleavage furrow.

When the advance of the furrow nears completion the nuclei in the two resulting blastomeres approach a central position and the subcortical currents progressively diminish. The insweep of the surface of the furrow does not accomplish complete separation of the two blastomeres; a narrow connecting stalk remains which finally breaks by subsequent, dislocatory movements of the two blastomeres.

(This article is based upon a lecture given at the Marine Biological Laboratory on August 5.)

STUDIES OF THE FACTORS INFLUENCING REGENERATION

(Continued from Page 125)

has repeatedly spoken against the rigid determination of structures in the embryo and adult and has demonstrated how structures can be modified at all times during the life history. The general experience of all of these early investigators was that the cells of the adult could be returned to an embryonic condition by cutting off a part of an organism or by starvation or other adverse conditions in the case of the entire organism. Thus the fact that cells in the adult may be so modified as to become equipotential was adequately demonstrated in the early nineteen hundreds. What is not clear, however, is the mechanism which brings about this modification. To be sure it is easy to cut off a part of an organism and have the remainder form a new part but if we knew the exact nature of the stimulus which is provided by cutting, we would have a marvelous tool for treating tissues which apparently do not react to the simple stimulus of cutting. Many animals have very limited powers of regeneration.

This paper will undertake to give therefore, a resumé of several summers' work by myself and some of my students on the factors concerned in the regeneration of the hydranths of Tubularia. I want to begin by showing how the hydranth regenerates under a given set of conditions. Tubularia is essentially a cylindrical tube of ectoderm and entoderm covered by a thin, tough perisarc. The hydranth differentiates within this perisarc and when fully formed emerges from the opening. This simple morphological pattern allows us to carry out many experiments which are impossible or difficult in other forms.

First we have to test the potencies of various regions of the stem by isolating the parts by cutting and by appropriate ligatures. By this means we see that the more distal regions of the stem regenerate a great deal faster than the more proximal regions. There is thus a gradient of inherent rates of regeneration along the stem.

The size of the stem also plays a part in determining rate. For these experiments the level of the regenerate must be kept constant and the length of the stem varied by ligatures tied at different levels.

By utilizing these two factors for determining rate we are able to study the competition of *two* regeneration regions in the *same* stem. We can take two regions possessing different rates and see how they partition the effect of adding length to each. To do this we first isolate the two regions by means of a ligature and then in another set of stems we can allow them to regenerate together. This material thus offers a unique opportunity to study the interaction of two growth centers, a phenomenon which is present in almost all biological systems. The results of these experiments show that when the stem is long the regenerating ends partition the effect of adding more stem to the system. When the stem is short the distal end takes all.

The stem behaves as if there was some internal factor in the cells responsible for the graded rates of regeneration. If this factor were a substance we could say it was present in graded concentration being highest at the distal end and lowest at the proximal end. We might even suggest that this substance was of the nature of a catalyst involved in the reactions which result in differentiation of a hydranth. But there is another factor which appears when we add more stem to the regeneration region. Something travels from resting region of the stem to the regenerating end and hastens regeneration. The most obvious way it could do this is through the circulation. Let us call this factor *S* assuming it to be a substance.

If this substance *S* circulates and is necessary for regeneration then we ought to be able to prevent the distal end from taking all of it by blocking the circulation within the stem. This has been done by injection of an oil droplet into the cavity of short stems. In the controls no proximal hydranths regenerate but when oil is injected they are able to do so.

There has been another suggestion by Lund, Child and others that dominance is brought about by a difference in electrical potential between the growing region and less active regions. My own experiments using Tubularia, Pennaria and Eudendrium do not leave me satisfied with this explanation. In the first place, there is no reason to believe that any current is flowing through the stems in sea water. Any difference of potential would simply cause a current to flow through the seawater and the potential difference would drop to zero. Secondly the potential differences are very small, usually less than a millivolt, and appear to be the result of polarity rather than the cause of it. Finally, even assuming the potential to be real and the current flowing, it is difficult to explain the mechanism of the effect of the electric current, since in *some* forms the growing region is *positive* while in other forms it is *negative*.

The first phase of this work may be summarized by saying that the isolated piece of the stem of Tubularia behaves as if we had two regions of different rates of regeneration partitioning available substances necessary for the differentiation of the hydranth.

The next phase deals with the *stimulus* for regeneration, namely why does a hydranth form at a cut surface or conversely what inhibits hydranth formation in the intact stem. Some years ago

Morgan placed one end of Tubularia in sand and found that no regeneration occurred, suggesting lack of O_2 as the cause. I repeated these experiments in a little different way by placing the cut end of the stem inside a glass tube. We find complete inhibition of regeneration of the end placed in the glass tube. The results are a bit surprising when you consider that in one experiment the end of the stem was almost flush with the opening of the tube. The experiments pointed strongly to low O_2 inhibiting regeneration. It occurred to me that low O_2 might likewise be the factor inhibiting differentiation of the hydranth from the intact stem. That is, the *perisarc* might prevent free gaseous exchange between seawater and the stem and so inhibit regeneration acting just as a glass tube. As a matter of fact, I had noticed in other experiments that when the stem was withdrawn into the perisarc for a few millimeters no regeneration took place.

This suggested two sorts of experiments. If O_2 tension was important in determining the differentiation of a hydranth, then varying the O_2 tension ought to inhibit and stimulate the process. Further if the perisarc was blocking free transport of O_2 to the tissues, removal of a small piece of the perisarc ought to allow the exposed tissue underneath to differentiate.

The first experiments were carried out by placing short stems in O_2 of varying tension and the rate of regeneration was measured. It was found that increase in O_2 above that of sea water hastened regeneration while decrease of O_2 brought about a decrease in rate until complete inhibition resulted.

It remained to be seen if the perisarc was blocking regeneration and this was shown to be the case by Mr. Zwilling. He removed small areas of the perisarc exposing the tissue to sea water, and found that the stem on both sides of the opening would form a hydranth. Since the stem was not cut these hydranths were attached to one another by their oral ends and emerged from the opening together. For this experiment the cut ends must be ligatured else they exert dominance over the middle regions of the stem. Mr. Zwilling's experiments show more clearly than the work on O_2 tension that exposure of the tissues to O_2 is probably the stimulus for differentiation of the hydranth. Injury to the tissue has nothing to do with the process. The ectoderm may or may not be cut in the act of removing the perisarc and regeneration still occurs. However, if the perisarc is replaced after the operation regeneration is suppressed.

It is rather interesting to speculate on how general this phenomenon is. Whether for example other tissues which do not differentiate when cut might be stimulated to do so by application of high O_2 tension. At any rate an adequate supply of O_2 must be one of the conditions for regeneration. The amount of oxygen necessary for regeneration is apparently greater than that necessary for maintenance. Stems which are tied off at both ends will live but will not regenerate unless an opening is made in the perisarc.

The next experiments were suggested by the work on O_2. The questions which arose were: (1), does the perisarc slow up penetration of oxygen so that ligatured stems consume less oxygen than open stems; (2), is the oxygen consumption of stems dependent upon oxygen tension?

For the first question one set of stems was tied off at both ends so that no regeneration occurred; another set was not ligatured which allows the distal end to regenerate; and, finally, a third set was ligatured in the middle of the stem so that two ends regenerate. It seems clear from the results that stems with open ends use more O_2 than ligatured stems also that the regenerating region is using more O_2 than the resting stem.

That the open ends are probably utilizing more oxygen than the middle of the stems is shown by a comparison of long and short stems. For an increase of three times in size we find only an increase of two times in oxygen consumption. If all parts of the stem were respiring at the same rate we should of course find a proportional increase in O_2 consumption with size.

Since increase in O_2 tension causes an increase in rate of regeneration and low tensions inhibit, the effect of varying oxygen tensions on the oxygen consumption of the stem was determined. Four gas mixtures were used. O_2, air, air and nitrogen, and nitrogen.

The oxygen consumption of stems does vary with oxygen tension and the rates of regeneration of these same stems was measured after removal form the manometers and a comparison of the effects of O_2 tension on regeneration and on oxygen consumption made.

There is a rough parallelism which is not exact as it is not possible to measure rate of regeneration accurately at low O_2 tensions. Some of these stems will begin to regenerate and then dedifferentiate back to stem. There is no way of including this type of regeneration in with the stems that form hydranths. The values for regeneration are, therefore, too low at very low tensions.

The beneficial effects of high O_2 can be further illustrated by some experiments of Mr. Moore where old stems of Tubularia were stimulated to form new hydranths by bubbling O_2 through sea water. The hydranths of these stems had previously been lost, and the stem had entered into a resting state. In running sea water these stems fail to regenerate but can be induced to do so by high O_2.

The experiments in this paper bear out the work of Child on the determination of polarity in

Corymorpha by differential exposure of short pieces of the stem. When these stems are allowed to rest on the bottom of a container the hydranth differentiates from the upper exposed surface. If the pieces are turned over so that no differential is maintained no hydranth differentiates but partial forms result with no polarity. A greater availability of O_2 at the exposed surface would explain these experiments and this was suggested by Child.

The work dealing with the effects of oxygen may be summarized as follows. When both ends of a stem are ligatured no regeneration takes place and oxygen consumption is low because the oxygen must diffuse through the perisarc to reach the tissues. If the end is left open more O_2 reaches the tissue at the end, an increase in O_2 consumption results and regeneration can take place. If both ends are ligatured and an opening is made through the perisarc at any point along the stem regeneration will occur on both sides of this point due to increased availability of oxygen. It appears to me then that the sole stimulus for the differentiation of the hydranth in Tubularia is an increased availability of oxygen. However, the tissues cannot continue to differentiate if a second more actively regenerating region is competing and exerting dominance. It would be extremely interesting to see how these two conditions could be applied to other organisms where the tissues do not regenerate new structures so readily. In many organisms the process of healing, by which a protective coating of some sort forms over the wound, would seem to prevent regeneration. If wounds could be kept moist and bathed with high O_2 supply it might allow for regeneration in those forms where the process appears to be limited.

(This article is based upon a lecture given at the Marine Biological Laboratory on August 12).

SOME PROBLEMS OF DISTRIBUTION AND VARIATION IN THE HAWAIIAN TREE SNAIL *ACHATINELLA*

d'Alte A. Welch
The Johns Hopkins University

The Hawaiian tree snail, *Achatinella*, from the Island of Oahu, with its many color patterns has been studied for many years. Gulick in 1872 was one of the first to show that these tree snails varied in color and form in different valleys. Pilsbry and Cooke later in 1912-1914 wrote a very complete monograph of *Achatinella* and showed that different color forms occurred on different parts of a ridge. But whether different color patterns occurred at random in isolated places, or whether there was some order in distribution, was not determined for lack of material.

Dr. C. M. Cooke of the Bishop Museum in Honolulu made a start towards working out the problem by collecting in highly restricted localities of not over three hundred square feet in extent, and encouraging others to do the same.

During the past six years much work has been done, not only collecting in small areas in the mountains, but plotting restricted localities on a map. The large advance sheets of the United States Geologic Survey Topographical maps of the Island of Oahu were used. Not only was new material plotted but also the approximate location of places visited by former collectors.

As many as possible of these plotted localities were checked and errors eliminated. Much still remains to be done on the Island of Oahu, the only place where *Achatinella* are found, concerning the precise location of localities and races. The problem is of large proportions because of the great area to be worked over, the ruggedness of the terrain, and the great difficulty in obtaining large series of *Achatinella* in most regions.

The main problems which have been investigated are as follows:

1. Do tree shells vary at random in nature or is there any order in the way the different color patterns are distributed throughout the mountains?

2. Do tree shells differ in size with altitude, and is there any correlation in size with altitude or humidity?

3. Is there any size change between the shells collected twenty years ago and the shells collected today from definitely known localities of limited extent?

The Island of Oahu contains two mountain ranges. The largest is the Koolau Range, which is about fifty miles long, and the other is the Waianae Range, approximately thirty miles long. To give some idea of shell variation with altitude, and the valley to valley pattern difference, I shall consider the variation of *A. apexfulva* on two ridges in the Koolau Range.

One of these ridges is the Kaukonahua-Poamoho Ridge. On this ridge *A. apexfulva* is found to have different color patterns in different areas at different elevations. The areas are made up of a group of localities of limited extent having similar color patterns. Starting at the lowest elevation areas the shells change from streaked forms to non-streaked forms. In the lowest area the color pattern is a dark bluish shade, above which

is a race of pinkish shells. These in turn grade to an area of bluish streaked forms. Then non-streaked grey shells with a yellowish ground come in which in turn change into pink forms. White shells may also occur here and all manner of intergrades between white and pink are to be found. At a still higher elevation the shells are all white finely lined with flesh color.

On the next ridge north, or on the Poamoho-Helemano Ridge the lowest shells are also bluish grey, but slightly different; the next higher area has another bluish grey streaked form with a brown sutural band; above these are areas of grey, black, dark pink banded, and finally pink shells.

Shell color patterns therefore vary from ridge to ridge and from valley to valley and there is a general trend towards a zonal elevation distribution. Streaked patterns usually go up to a definite elevation; then non-streaked forms occur, while the highest localities generally have whitish forms.

These color zones are present on all the ridges in the Koolau Range from which material has been obtained. They seem to occur at definite distances from the backbone ridge and do not appear to be correlated with any specific elevation. The same shade of color pattern however does not always occur at a definite distance from the backbone. In one section covering several ridges yellow patterns occur, while further to the north at approximately the same distance from the backbone several other ridges will have brown or bluish grey color patterns. Thus the occurrence of any particular colored shell in any given zone is a random affair and not predictable. However streaked patterns appear mostly in the lower elevation zones, while non-streaked forms occur in the higher elevation zones. The last two highest zones within about two miles of the backbone usually have white color patterns.

All this applies only to *A. apexfulva*. Another species would show an entirely different altitudinal and valley to valley variation.

At present I am still working on *A. apexfulva* and have not worked out as yet the size variation with altitude. But in a paper now in press entitled "The Variation and Distribution of *Achatinella mustelina* in the Waianae Mountains," this problem has been studied.

About 582 localities were collected and plotted in the Waianae Mountains. The shells from each locality were graded by being measured to the nearest millimeter, and the mean length was obtained for each locality. In the northern section of the Waianae Mountains the shells decrease in mean length with altitude from about 20-21 mm. to 18 mm. at higher elevations. Farther to the west on the northern side of the mountains there is no zonal differentiation and localities of 18 and 19 mm. are found scattered at random. In Keaau Valley very large shells over 23 mm. mean length are found, while at the same elevation in the adjacent Valley of Makua the shells are 20 or 19 mm., depending on the proximity to the backbone. West of Mt. Kaala 17 mm. shells occur; the farther away the locality is from this region, the larger are the shells. To the south even at higher elevations the shells are 20-21 mm., and coming north we have a sector of 19 mm. shells.

It seems obvious then that altitude is not the controlling factor in shell size, because at the same altitude shells of different lengths occur.

Mt. Kaala is the highest peak and has the greatest amount of precipitation of any place in the Waianae Mountains, ranging from 86 to 99 inches per year. At sea level the range of precipitation is from 18 to 22 inches per year. The southern portion of the Waianae Mountains has no rain gauge stations but is much dryer than the northern section of the mountains. The region below Mt. Kaala where small 17 mm. shells occur is probably the wettest region of the mountains because the prevailing west winds carry the clouds over to the Waianae Mountains from the Koolau Range and hit this peak 4000 feet high which is the highest one in the mountains. More water would be expected to fall on this side. The farther away from this region the dryer the localities become. Therefore there is a correlation between size decrease and increase of moisture conditions.

Again it must be noted that this only applies to the species *A. mustelina*, for the reverse is true in other forms.

In several localities in the Waianae Mountains I was able to obtain series of shells which had been obtained from the same locality in different years over a period of 10 or more years. The localities were all restricted ones and definitely located on a map. In all these localities no marked size variation was noted in the shells collected 10 years ago and the shells collected to-day. It must be stated however that I only roughly graded my shells to the nearest mm., but I think that if any marked increase or decrease of shell size occurred it would have shown up. This does not agree with Crampton's findings in his work on the *Partula* of Tahiti, and Moorea where he obtained a decrease in size between the shells he collected at one time in a valley and those collected in the same valley 10 years later. But it must be remembered that he was dealing with an entirely different genus so that what I found in *Achatinella* may not at all apply to *Partula*.

In summary, tree shells on the Island of Oahu are found to vary not only from valley to valley but also show marked color differences in different zonal areas at different elevations. One

species varies in a definite way, while another has an entirely different way of varying.

Achatinella apexfulva tends to have streaked patterns in the lower zonal areas, and non-streaked patterns in the higher zones. The very highest zone usually contains white color patterns.

In the species *A. mustelina,* correlations exist between shell size and moisture conditions, the shell decreasing in size with increase of moisture conditions.

No size change is noted between the shells collected to-day and the shells collected in the same restricted locality 10 years ago.

In conclusion I wish to say that all this work is a start towards working out some of the basic problems of shell variation and distribution. The present work has been mainly to find out what the picture is, not to find out how it came about.

(This article is based on a seminar given at the Marine Biological Laboratory on August 2.)

NEW MICRO-ENZYME TECHNIQUES FROM LINDERSTROM-LANG'S LABORATORY

Dr. David Glick
Beth Israel Hospital, Newark, N. J.

The fine techniques developed by Drs. K. Linderstrøm-Lang and Heinz Holter of the Carlsberg Laboratory in Copenhagen, which enable studies of enzymes and certain elements in microtome tissue sections, have been extended to yield further refinements.

Linderstrøm-Lang has evolved two new techniques which allow investigations to be made directly upon single cells and even parts of cells.

One depends upon the volume change accompanying enzyme reactions: A drop, containing enzyme substrate and buffer, may be placed in a tube containing a mixture of bromo-benzene and kerosene in which the proportion of bromo-benzene is greater in the lower portion of the tube so that a density gradient is obtained. The drop will come to rest at a certain level, then as the action proceeds it will move due to the volume change occurring. The extent of the reaction is proportional to the magnitude of the drop's excursion. The distance moved may be observed with a cathetometer or an occular with scale markings.

The other technique is based upon the principle of the Cartesian diver. A small diver with a total volume of about 10 cmm. is used as the reaction vessel and any gas change occurring may be followed by observing the pressure on a water manometer needed to bring the gas volume to a fixed value, i.e. the pressure required to bring the diver to a definite position in the tube of liquid in which it is immersed. Thus the diver acts as a constant volume Warburg apparatus. The refinement that this procedure allows may be comprehended when it is realized that over 10,000 determinations of choline esterase activity may be made upon a ganglion weighing 10 mgs.

Linderstrøm-Lang and Glick developed a method for estimation of choline esterase by means of these divers, and Glick applied the method to a study of the question of nerve impulse transmission by acetyl choline. The enzyme activity in the superior cervical ganglion of the cat was found to be such that the acetyl choline liberated by a single impulse could be destroyed in the theoretically least time of 0.015 m.sec. This is less than 1% of the refractory period of 2 m.sec. found by Brown. However, were the enzyme to act under the most unfavorable conditions possible the time would be over 8 sec. Between these two extremes, the condition for destroying the acetyl choline within a time comparable to the refractory period may be calculated. This condition would be the localization of the enzyme and sustrate in about 1/420 of the total ganglionic volume. Hence, it may be seen that the choline esterase within the ganglion can hydrolyse the acetyl choline liberated by a nervous impulse in a time comparable to the refractory period, as Dale's theory postulates, if a relative localization of the enzyme and acetyl choline exists.

(This report is based upon an informal conference held by Dr. Glick in the Old Lecture Hall on July 28.)

Dr. P. S. Henshaw who has been biophysicist at the Memorial Hospital for the Treatment of Cancer and Allied Diseases in New York for almost nine years in giving up that position to become senior biophysicist at the new National Cancer Institute at Bethesda, Maryland, just outside Washington, D. C. The new institute will be a part of the large health center being developed by the U. S. Public Health Service at Bethesda. While Dr. Henshaw joined the institute staff on August 1, he will continue work at the Memorial Hospital until about January 1 at which time he will go to Washington and probably be associated with the radiation division of the U. S. Bureau of Standards until the construction of the institute building is completed.

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris Gorokhoff and Hazel Goodale.

Entered as second-class matter, July 11, 1935, at the U. S. Post Office at Woods Hole, Massachusetts, under the Act of March 3, 1879, and re-entered July 23, 1938.

Introducing

Dr. Stephen Karady, Assistant Professor of Internal Medicine, Franz Josef University at Szeged, Hungary; Rockefeller Foundation fellow at McGill University, Montreal and the Marine Biological Laboratory.

Born at Jaszbereny, Hungary, Dr. Karady received his early education in Budapest and obtained his doctor's degree at the Medical College of the Pazmany Peter University of Budapest. He received a scholarship to study half a year at Vienna and later another half year at Berlin. As a student he did research in anatomy and pathology and clinical work in internal medicine, and became an assistant in internal diseases and neurology.

Results of research which Dr. Karady has done are recorded in 38 publications which deal with the mechanism of alimentary hyperglycemia, of uremia, of insulin shock, and cardiazol shock; also with the pathogenesis of sclerosis multiplex, the mineral metabolism of the organism, the physiology of adenosine, acetylcholine, choline, the problem of histamine-thyroxine antagonism, histamine-tachyphylaxis and histamine resistance.

During the past few years Dr. Karady's work has dealt mostly with the rôle of histamine in the organism under physiological and pathological circumstances, as especially in the heteroprotein therapy, in the X-ray cater, in the experimental and organ anaphylaxis, in pregnancy, in hypochloremic uremia, in surgical shock, etc. He has discovered how to improve the course of X-ray cater, anaphylactic and allergic diseases, (such as asthma and hay fever) by the means of histamine treatment.

The most important part of his work deals with surgical shock. A method has been worked out by which it is possible to detect before an operation those patients who are sensitive to surgical shock under even minor operative procedures. After long experiment, a treatment was also found to prevent the tendency to surgical shock.

In Montreal further investigation made clear the mechanism of surgical shock; a series of experiments were made on the histaminase content of the normal and diseased organism particularly in ways which involved the endocrines. Much work was also done on the alarm reaction, and on the question of existence of "endantigen."

In Woods Hole Dr. Karady has been studying the problem of anaphylaxis and alarm reaction on lower vertebrates using for experimental animals *Fundulus majalis* and *heteroclites*. The experiments are not yet finished but they are showing interesting results which will be published later.

Scientific pursuits do not entirely fill the day for this investigator; Dr. Karady finds time for sailing, excursions on the *Winifred*, golf and many hours of tanning at the beach. Next week he leaves for Rochester, Minnesota, where he will visit the Mayo Clinic. When his work at McGill is completed this fall, he will return to Szeged.

—*M. F. M.*

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ITEMS OF INTEREST

DR. ALFRED HUETTNER, who has been professor of biology at Washington Square College, New York University, has resigned in order to assume a new position as associate professor of biology at Queens College, Long Island.

DR. RICHARD WEISSENBERG, formerly professor extraordinarius of anatomy at the University of Berlin (Germany) and last year visiting professor of cytology at the School of Medicine of Washington University, St. Louis, has been appointed a fellow at the Wistar Institute of Anatomy and Biology and is undertaking research at the Effingham B. Morris Biological Farm of the Wistar Institute at Bristol, Pa.

GARDNER EMMONS of the Marine Division of the United States Weather Bureau has been appointed assistant professor of meteorology at New York University where he will continue his work on synoptic meteorology. He has been associated also with the Woods Hole Oceanographic Institution.

MISS ELIZABETH REEDER, who took the embryology course at the Marine Biological Laboratory in 1935, is to be instructor in biology at Sweet Briar College. She took her Ph.D. degree at the University of Missouri this June.

DR. DANIEL MAZIA has been appointed instructor in zoology at the University of Missouri. He will assume his duties there in September.

MR. B. COMMONER has been appointed assistant in biology at Harvard University.

DR. O. E. SCHOTTÉ left Woods Hole on Wednesday for a three day visit to Amherst College, where he is professor of biology, to complete work on a manuscript.

DATE OF DEPARTURE OF INVESTIGATORS

Ballard, W. W.	August 11
Birnbaum, W. Z.	August 5
Black, E. C.	August 4
Churney, L.	August 1
Donnellon, J. A.	August 11
Fox, E.	August 11
Funkhouser, Elizabeth M. J.	August 11
Harris, D. L.	August 9
Hodge, C.	August 3
Hunninen, A. V.	August 11
Lewis, Lena	August 4
McCann, L. P.	August 11
Miller, Jane	August 11
Patrick, Ruth	August 3
Root, R. W.	August 11
Salzburg, F. P.	August 11
Sisson, W. R.	August 10
Walzl, E. M.	August 11
White, Elizabeth C.	August 3

Several workers at the Marine Biological Laboratory attended the sixteenth International Physiological Congress, which convened in Zürich, Switzerland, during the present week. It was preceded by the Fifth International Congress of Experimental Cytology, and will be followed by an International Veterinary Congress from August 21 to 25. Premier Juan Negrin, of loyalist Spain, attended the Physiological Congress.

At the *Staff Meeting* of the Woods Hole Oceanographic Institution on August 18, two papers were presented: "The Multiplication of Bacteria in Stored Sea Water" by D. Q. Anderson, and "Phytoplankton in the Gulf of Maine" by Lois C. Lillick.

DR. GEORGE CLARK of Harvard University and the Woods Hole Oceanographic Institution has been given a leave of absence from the latter institution for three months to study the techniques used in fresh water lakes with a view to determining their application to marine conditions. Dr. Clark is now with Dr. Juday who is director of the Trout Lake Limnological Laboratory in Wisconsin.

DR. HERMAN YAGODA, Chemist for the United States Customs Service in New York City, came to Woods Hole with his wife and baby daughter, Beryl, on Monday for a month's visit. Dr. Yagoda was formerly Baker Fellow in American Chemistry at Columbia University and has come to Woods Hole to confer with Dr. Chambers and Dr. Rakestraw.

DR. GEORGE M. MCKINLEY, instructor in biology at the University of Pittsburgh, has been promoted to the rank of assistant professor.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
August 20	11:56	
August 21	12:41	12:59
August 22	1:40	1:56
August 23	2:43	2:58
August 24	3:35	3:53
August 25	4:30	4:46
August 26	5:20	5:36
August 27	6:06	6:31
August 28	6:54	7:15

In each case the current changes approximately six hours later and runs from the Sound to the Bay.

ITEMS OF INTEREST

Miss Louise Gregory, professor of biology at Barnard College is spending a few days in Woods Hole. She is the guest of Dr. and Mrs. G. N. Calkins.

Mr. J. F. Seybolt, of Yale, who has been at Woods Hole is at present at the Cape Cod Hospital, Hyannis, where he underwent an operation for appendicitis.

Final Tennis Tournament Scores

Men's single. Semi-final round, Hekhuis defeated Frank, 6-3, 7-5; Miller defeated Rugh, 6-1, 6-2. Final round, Miller defeated Hekhuis, 6-1, 6-2.

Junior singles. Semi-final round, Ted Jones defeated Tom Garlock, 6-0, 6-0; Joe Crossley defeated C. Schmidt, 6-3, 8-6. Final round, Ted Jones defeated Joe Crossley, 6-4, 6-2.

Dr. Raymond L. Barney, Professor of Biology at Middlebury College, died on July 12. Before becoming associated with Middlebury College, Dr. Barney was Director of the laboratories at Beaufort, North Carolina and the Fairport Laboratories in Iowa.

Jowain Cattell, 3 year old adopted daughter of Dr. Psyche Cattell, was killed on Monday afternoon by a fast-moving automobile on the road to Nobska Beach.

The program selected for the phonograph concert to be presented at the Clubhouse at eight o'clock Monday evening, August 22, is as follows: Der Freischütz Overture, Weber; Symphony No. 4, Schumann; Symphony No. 1, Brahms; Verklaürte Nacht, Schoenberg.

INVERTEBRATE CLASS NOTES

What might have looked to a casual observer like a man overboard as the *Winifred* hove to near Kettle Cove Friday was merely a fellow invertebrate who had decided not to wait for one of the small rowboats bouncing up and down on a very choppy sea to ferry him to land. Several others followed his example and before they had spanned the hundred odd yards separating the boat and shore began to wish they hadn't ever started. They had it all over the poor unfortunates who didn't scorn the boats though; they didn't get quite so wet as the latter, who were thoroughly drenched by every wave that washed over the sides, threatening to swamp it hook, line and sinker; they didn't have to make the perilous descent from the *Winifred* or *Nereis* that managed to be rolling disastrously out of phase with the struggling little boats.

After everyone finally reached the shore, collecting went on apace with Dr. Bissonette's group finding choice specimens and leaving them near the surface for others to turn up and crow about.

After lunch-time had passed unheeded by the powers that were for what seemed hours, Dr. Bissonnette and his team were seen on the top of a hill surveying the country—for all the world like Balboa and his followers about to cross the Isthmus of Panama. "Company, forward march!" and they were off across the island of Naushon to Tarpaulin Cove followed closely by a group of starving invertebrates. As time went on, what had looked on the map like a half mile walk lengthened into at least five miles as we went over hill and down dale, fraternizing with wood ticks and scaling stone fences with crystalizing dishes in our hands. For a while it looked as though we would never get to the other shore of the island and, what was more important, the *Winifred* with our lunch aboard. The impossible often happens, however, and we reached both our geographic and gastronomic goals.

The North side of the laboratory certainly showed the South side the way home on the baseball diamond last Saturday afternoon. A few partial umpires would have come in very handy but they were not to be found. In their absence the score attained the pitiful total of eighteen to five, and the South side retired vanquished but undefeated.

A strange and seemingly uncurable disease has hit practically the entire invertebrate class lately. Especially in the southwest corner of the laboratory and on team one during field trips is this fatal malady noticed. This state of affairs is particularly unfortunate because the patient is not the one to suffer; it is his poor companions who are pained most acutely.

Amphitrite ornata, Amphitrite brunnea, Thelepus cincinnatus, Pista palmata—these are the names reeled off by a poor bewildered student trying to identify a mystifying worm that can't make up his mind whether he's green or red, but who most definitely has a lot of prostomial tentacles and setae from the fourth to twentieth segment. Multiply this experience by twenty or so and you have the complete diary of an invertebrate zoologist for August 15.

A dredging trip in the hot sun, but with a smooth sea was almost a relief the following day. With no recorded cases of seasickness or sunburn, and with the dangerous epidemic of punning almost wiped out the day can be said to have been quite successful.—*Elizabeth L. Jordan.*

THE CHORAL CLUB CONCERT

Anne Nathan Meyer

When a Choral Club gives its twelfth annual concert, it is certainly entitled to be called an institution. And it is an institution of which Woods Hole may well be proud. I wonder if many of our residents realize the terrific handicaps under which it exists. Of the thirty-three members whose names appeared on last night's program, only five had joined the first year. One or two more belonged very shortly after, but most of the singers are new. To an old member of the audience like myself, there is something peculiarly interesting, even if it is somewhat disconcerting, to see the sons or daughters standing on the platform where their fathers or their mothers stood before. Two families should receive praise—the Lintons and the Masts for furnishing members of two generations who sing together.

The Chorus meets only twice a week for a few weeks. Probably it is never possible for every member to rehearse at any one time (except, perhaps, the panicky few days before the actual concert), and it is necessary to begin one of the bi-weekly rehearsals at nine-thirty at night when most of us respectable workers are more concerned with thoughts of slumber than songs by Handel, Gretchaninoff, Brahms or any other great master.

In short the splendid results achieved are nothing less than magic. I honestly believe that no one else except that magician Ivan Gorokhoff could turn the trick. He knows just what he wants, he has a clear idea in his head of his objective and has the skill, the knowledge, the patience and also the enthusiasm to attain it. That isn't saying that the perfection that is undoubtedly in the heart of so great a leader is accomplished, but that it aproaches it at all as it does, deserves the heartiest thanks and appreciation of all of us, to the hard-working leader and to each and every member of the devoted group.

Make no mistake! They work hard, but they enjoy it. Ask any member and note how unmistakably they express the sense of the fun it is. All honest work is fun. The only truly dull life is that in which earnest purpose is absent.

And the audience had fun. I was sorry to see that some of the old stand-bys were absent, yet the small hall was filled almost to capacity and the applause was eager and sincere. The program was more than usually international, and even if the familiar Arkhangelsky seemed more exquisitely done than any other, that much may be allowed to our Russian maestro. The Brahms gypsy song was sung with tremendous fervor and a lilting swing that made one aware that at least some of New England's traditional inhibitions had considerably loosened. The leader's daughter, who has grown up before my eyes, did admirable work at the piano in securing the temperamental interpretation that it needed.

There was zest, too (and in these parlous times let us be prayerfully thankful for it!) in the singing of the Cuckoo Song and the rollicking lilt of the old English Air. Several of the numbers had to be repeated, they were so enthusiastically received.

PENZANCE PLAYERS TO PRESENT ANNUAL PERFORMANCE

Mystery and romance will walk the stage of Community Hall next week as the Penzance Players present their annual production, "Not Herbert," by Howard Irving Young, on Wednesday and Thursday, August 24 and 25.

The fast-moving plot deals with the mysterious poet son of broker Alden and with the family jewels, which change hands several times before the exciting climax. The comedy, whose locale is in New York, consists of four acts and is full of amusing lines.

The Penzance Players are being directed by Mrs. Gardiner Handy, who has supervised several previous productions of the organization. The receipts will be used for the first time to allow the players to make a start toward owning their own stage equipment.

Tickets are on sale for $1.10 and 55 cents. The show will begin at the Community Hall at 8:30 P. M.

The cast which has been selected for the play is as follows: Herbert Alden, Tom Cross; Tracy Sutton, George Compton; Stephen Alden, Herbert's father, Garret McClung; Fletcher, Bill Cross; George, Jimmy Wright; Jim, Edmund Mayo; Fanshaw, a detective, Dick Cross; Ruth Webster, Margery Mitchell; Cynthia Alden, Herbert's sister, Betty Copeland; Polly, Mary Meigs; Bertha Alden, Herbert's mother, Edith Mitchell; Mrs. Blaine, Camilla Riggs.

A severe thunder storm which occurred early Wednesday morning resulted in damage to the Homestead building of the Marine Biological Laboratory which was struck by lightening. The bolt past through a window and roof of the southwest corner of the building ripping out a side window casing and tearing pieces from the roof. Miss Theresa McGee, a teacher who works at the laboratory mess hall, was sleeping in the room and the electric charge passed within a few feet of her head. Fuses were blown out not only in the Homestead but in the Old Main Building, the Hubbard House and the bungalow where the Rugh family lives. The Kidder house was also struck by lightening but no damage was done.

THE COLLECTING NET

Vol. XIII, No. 7 SATURDAY, AUGUST 27, 1938 Annual Subscription, $1.50 Single Copies, 30 Cents.

THE INFLUENCE OF FLUCTUATIONS IN THE MAJOR OCEAN CURRENT ON THE CLIMATE AND THE FISHERIES

C. O'D. Iselin
Assistant Professor of Oceanography, Harvard University

The relatively few physical oceanographers in the world would like nothing better than to be given time to develop the technical side of their studies, for there is a great deal still to be learned about the circulation of the ocean. However, of recent years this very young field of science has made good progress and as a result, there is increasing pressure on the hydrologist to put his investigations to some practical use. Would it not be possible, for example, by studying the Gulf Stream as it flows past this coast to predict a mild winter for northern Europe? Can the failure of a given year class of commercial fish sometimes be explained by sudden off shore movements of the coastal waters and are not these perhaps caused by variations in the strength of the main, deep oceanic currents?

It is such questions (*Continued on page* 166)

M. B. L. Calendar

TUESDAY, August 30, 9:00 A. M.
General Scientific Meeting: Partial list of papers may be found on page 164).

WEDNESDAY, Aug. 31, 9 A. M.
General Scientific Meeting (cont.): Partial list of papers may be found on page 164).

THURSDAY, September 1, 8 P. M.
Lecture: Dr. H. J. Muller: The remaking of chromosomes.

FRIDAY, September 2
Genetics Society: 8:00 A. M., Demonstrations and exhibits (Old Lecture Hall). 10:00 A. M., Reading of papers.

SATURDAY, September 3
Genetics Society: 8:00 A. M., Demonstrations and exhibits (Old Lecture Hall). 10:00 A. M., Reading of papers.

THE SUMMER MEETING OF THE GENETICS SOCIETY OF AMERICA AT THE MARINE BIOLOGICAL LABORATORY

Dr. P. W. Whiting
Local Secretary
University of Pennsylvania

This year, (September 1-3), as for the past four years geneticists will assemble at Woods Hole to read papers, discuss their problems and demonstrate their various methods of work and their materials. For relaxation they will enjoy a boat trip, swimming party and a clam bake at Tarpaulin Cove. Attendance at these meetings has been constantly growing since 1934 and this year promises to be even larger than last, if we may judge from the number of titles sent in for talks and demonstrations. The first year about 70 attended the clam bake at Sippiwisset, but since then boat trips have been taken to Tarpaulin Cove and this year there will be an all afternoon trip around the islands starting from the Eel Pond at two o'clock and arriving early at Tarpaulin Cove for a swim at the beach and a clam bake in the grove. Those who wish may omit the longer trip, leav-

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THE TRUSTEES OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY, PHOTOGRAPHED AT THE ANNUAL MEETING, AUGUST 9, 1938

Back row: H. B. Goodrich, W. C. Curtis, Lawrason Riggs, Jr., E. N. Harvey, L. V. Heilbrunn, B. H. Willier, Ivey Lewis, M. H. Jacobs, F. P. Knowlton, P. B. Armstrong.

Middle row: E. R. Clark, C. C. Speidel, W. E. Garrey, Charles R. Stockard, Ross G. Harrison, A. P. Mathews, Franz Schrader, C. E. McClung, Lorande L. Woodruff, Ralph S. Lillie, Caswell Grave, Laurence Irving, A. C. Redfield, S. O. Mast.

Front row: H. C. Bumpus, G. H. Parker, W. B. Scott, T. H. Morgan, E. G. Conklin, E. B. Wilson, Frank R. Lillie, Gary N. Calkins, W. J. V. Osterhout, Otto C. Glaser, D. H. Tennent, W. R. Amberson, Charles Packard.

ing Woods Hole at 4:30 p. m. direct for the clam bake. If several wish an early return, one boat may get them back by nine o'clock. All are welcome whether members of the Society or not. Tickets should be bought at the main entrance, Brick Building, on Thursday evening or Friday morning. In case of rain the clam bake will be held in the club house and there will be a rebate on the tickets.

This year demonstrations are scheduled early for both Friday and Saturday mornings at the Old Lecture Hall. It is thought that this time will be cooler in case of a hot day. Eight o'clock means that those who wish may start the day immediately after an early breakfast, but space will be available and those who prefer may continue discussion and demonstration as long as they wish. Sessions for formal reading of short papers will begin at ten o'clock both mornings.

Lectures of interest to geneticists have been held by the Marine Biological Laboratory on evenings before the meetings of the Society. This year Dr. H. J. Muller of radiation and mutation fame will tell us about remaking chromosomes and doubtless we will hear the latest conclusions about position effect, the nature of the gene, and whether there are any genes!

The complete program of the meetings is published this year as before, and this year THE COLLECTING NET is also publishing the abstracts of all the papers in the hope that biologists will avail themselves of the opportunity to read at leisure what geneticists have been doing and thus be prepared to ask questions and to better understand the exhibits.

PROGRAM OF THE SUMMER MEETING OF THE GENETICS SOCIETY OF AMERICA AT THE MARINE BIOLOGICAL LABORATORY, SEPT. 1 TO SEPT. 3, 1938

Officers of the Genetics Society of America

President, L. J. STADLER, University of Missouri, Columbia, Mo.

Vice-President, M. DEMEREC, Carnegie Institution of Washington, Cold Spring Harbor, N. Y.

Secretary-Treasurer, E. W. LINDSTROM, Iowa State College, Ames, Iowa.

Local Secretary and Representative, P. W. WHITING, University of Pennsylvania, Philadelphia, Pa.

Thursday Evening, September 1, 8:00 P. M., Auditorium

Marine Biological Laboratory Evening Lecture

H. J. MULLER, Institute of Animal Genetics, King's Buildings, Edinburgh, Scotland. The remaking of chromosomes.

Friday Morning Session, September 2, 8:00 A. M., Old Lecture Hall

Demonstrations and Exhibits

(Space will be available and time unlimited during the period of the meetings. Demonstrations 1-14 are scheduled for convenience on Friday morning and Demonstrations 15-23 on Saturday morning but members of either group may utilize either or both periods if desired. Friday afternoon is also available for those who do not care for the boat trip.)

(1) HAMBURGER, VICTOR, Washington University, St. Louis, Mo.: Transplantation of limb primordia of homozygous and heterozygous creeper embryos onto normal chick embryos.

(2) EASTLICK, HERBERT L., University of Missouri, Columbia, Mo.: A study of pigmentation in the chick embryo by means of limb bud transplantation.

(3) WOOLLEY, GEORGE W., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me., and COLE, LEON J., University of Wisconsin, Madison, Wis.: A new dwarf mutation (dw 2) in Rattus norvegicus.

(4) REED, S. C., McGill University, Montreal, Canada: Determination of hair pigments in the mouse.

(5) HOWARD, ALMA, McGill University, Montreal, Canada: Chromosome behaviour in relation to cancer susceptibility in mice.

(6) DAVENPORT, C. B., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: Studies in human genetics: Acquisition of specific head form.

(7) BURKS, BARBARA S., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: A case of autosomal linkage in man: Congenital tooth deficiency and hair color.

(8) STOCKER, GAIL, University of Pennsylvania, Philadelphia, Pa.: Mutant types and mosaics of Habrobracon.

(9) ANDERSON, R. L., Johnson C. Smith University, Charlotte, N. C.: Non-autonomous eye transplants in Habrobracon: methods and specimens.

(10) MARSHAK, ALFRED, New England Deaconess Hospital, Boston, Mass.: The stage of mitosis at which chromosomes are rendered less sensitive to x-rays by ammonia.

(11) WHITE, M. J. D., University College, London, Eng., and Columbia University, New York, N. Y.: Photographs illustrating the relationship between heteropycnosis and spiral structure of chromosomes.

(12-14) METZ, C. W., Carnegie Institution of Washington, Baltimore, Md.

(12) A recurrent spontaneous break in a salivary gland chromosome in Sciara.

(13) The nature and significance of minute chromosomal differences in species hybrids and in ordinary wild strains of Sciara.

(14) Structure of salivary gland chromosomes in Diptera.

THE COLLECTING NET was entered as second-class matter July 11, 1935, at the Post Office at Woods Hole, Mass., under the Act of March 3, 1879, and was re-entered on July 23, 1938. It is devoted to the scientific work at marine biological laboratories. It is published weekly for eight weeks between July 1 and September 15 from Woods Hole, and is printed at The Darwin Press, New Bedford, Mass. Its editorial offices are situated on Main Street, Woods Hole, Mass. Between June 1 and October 1 communications should be addressed to Woods Hole, Mass.; at other times they should be directed to THE COLLECTING NET, Garrison, N. Y. Single copies, 30c; subscription, $1.50.

Friday Morning Session, September 2, 10:00 A. M., Auditorium

Reading of Papers

(1) Payne, Nellie M., University of Minnesota, Minneapolis, Minn.: The "yellow" colors in Microbracon hebetor (Say) [Habrobracon juglandis (Wes.)] (10 min.)

(2) Whiting, P. W., University of Pennsylvania, Philadelphia, Pa.: Sex-determination and reproductive economy in Habrobracon. (10 min.)

(3) Carothers, E. Eleanor, State University of Iowa, Iowa City, Ia.: A hybrid Acridian gynandromorph. (15 min.)

(4) White, M. J. D., University College, London, Eng., and Columbia University, New York, N. Y.: A new type of anomalous meiosis. (15 min.)

(5) Metz, C. W., Carnegie Institution of Washington, Baltimore, Md.: Observations on the nature and significance of minute chromosomal differences in species hybrids and in ordinary wild strains of Sciara. (8 min.)

(6) Metz, C. W., Carnegie Institution of Washington, Baltimore, Md.: Structure of salivary gland chromosomes in Diptera. (7 min.)

(7) Koller, P. Ch., California Institute of Technology, Pasedena, Calif.: Chromosome variation in populations of Drosophila pseudoobscura from contiguous localities. (10 min.)

(8) Dobzhansky, Th., and Koller, P. Ch., California Institute of Technology, Pasadena, Calif.: Sexual isolation between two species of Drosophila—a study on the origin of an isolating mechanism. (15 min.)

(9) Kaliss, Nathan, Columbia University, New York, N. Y.: The expression of a short lethal deficiency in Drosophila melanogaster. (10 min.)

(10) Raffel, Daniel, Baltimore, Md.: A genetic analysis of apparent losses of the distal end of the scute 8 chromosome. (15 min.)

(11) Weinstein, Alexander, Columbia University, New York, N. Y.: The arrangements of the chromatids in crossing over. (15 min.)

Friday Afternoon and Evening, September 2

Excursion on the Boat *Winifred* starting at 2:00 p. m. Trip around the islands ending at Tarpaulin Cove for swim and clam bake.

Boat trip direct to Tarpaulin Cove starting from the Eel Pond at 4:30 p. m.

(Purchase tickets Friday morning at the main entrance, Brick Building. The same price, $1.60, covers boat trip and clam bake.)

An early return from Tarpaulin Cove arriving at Woods Hole at 9:00 p. m. may be arranged for one of the boats if desired.

Saturday Morning Session, September 3, 8:00 A. M., Old Lecture Hall

Demonstrations and Exhibits

(15) Singleton, W. Ralph, Connecticut Agricultural Experiment Station, New Haven, Conn. and University of Missouri, Columbia, Mo.: Cytological observations on deficiencies produced by treating maize pollen with ultra violet light.

(16-17) Buchholz, J. T., University of Illinois, Urbana, Ill., and Blakeslee, A. F., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.

(16) Direct demonstration of the result of crossing-over in the male gametophyte of Datura.

(17) Demonstration of gene types of pollen-tube growth in Datura stramonium.

(18) Blakeslee, A. F., and Avery, A. G., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: Induction of diploids from haploids by colchicine treatment.

(19) Hughes, Roscoe D., Medical College of Virginia, Richmond, Va.: The chromosomes in the hybrid between Drosophila virilis virilis and Drosophila virilis americana Spencer.

(20) Kaliss, Nathan, Columbia University, New York, N. Y.: The expression of a short lethal deficiency in Drosophila melanogaster.

(21) Bedichek, Sarah, North Texas Agricultural College, Arlington, Texas.: Sex balance in the progeny of triploid Drosophila.

(22) Kaufmann, B. P., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: An x-ray induced "reversed repeat."

(23) Poulson, D. F., Yale University, New Haven, Conn.: Polyploidy in eggs of Drosophila melanogaster carrying X-chromosome deficiencies.

Saturday Morning Session, September 3, 10:00 A. M., Auditorium

Reading of Papers

(12) Kimball, Richard F., Johns Hopkins University, Baltimore, Md.: A lag in the change of phenotype following a change in genotype in Paramecium aurelia. (10 min.)

(13) Eastlick, Herbert L., University of Missouri, Columbia, Mo.: A study of pigmentation in the chick embryo by means of limb bud transplantation. (15 min.)

(14) Landauer, Walter, Storrs Agricultural Experiment Station, Storrs, Conn.: Types of cross-beak in fowl. (10 min.)

(15) Warmke, H. E., and Blakeslee, A. F., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: Induction of tetraploidy in Nicotiana sanderae and in the sterile hybrid N. tabacum x N. glutinosa by colchicine treatment. (12 min.)

(16) Jones, D. F., Connecticut Agricultural Experiment Station, New Haven, Conn.: Variable effect of the C locus in maize following translocation. (15 min.)

(17) Mather, Kenneth, John Innes Horticultural Institution, Merton, Eng.: Chiasma frequency in trisomic maize. (15 min.)

(18) Sax, Karl, Arnold Arboretum of Harvard University, Boston, Mass.: The effect of x-rays on chromosome structure. (15 min.)

(19) Loehwing, W. F., State University of Iowa, Iowa City, Ia.: Physiological factors involved in differentiation of male and female parts of the angiosperm flower. (15 min.)

(20) Schweitzer, Morton D., Cornell University Medical College, New York, N. Y.: Role of heredity in childhood rheumatism. (15 min.)

(21) Dove, Wm. Franklin, University of Maine, Orono, Me.: A study of individual versus group needs as a theoretical and an experimental approach to aggridascendance. (15 min.)

(22) Jollos, Victor, Madison, Wis.: Further tests of the role of cosmic radiation in the production of mutations. (15 min.)

(23) Boche, R. D., Carnegie Institution of Washington, Baltimore, Md.: Hymenopteran parasitism of Drosophila.

(24) Buck, John B., Carnegie Institution of Washington, Baltimore, Md.: Structure of living salivary gland chromosomes.

(25) Margolis, Otto S., Western Reserve University, Cleveland, Ohio.: The effects of reduced atmospheric pressure and oxygen on facet number in Bar-eyed Drosophila.

ABSTRACTS OF PAPERS PRESENTED AT THE 1938 SUMMER MEETING OF THE GENETICS SOCIETY OF AMERICA AT THE MARINE BIOLOGICAL LABORATORY, WOODS HOLE, MASS., SEPTEMBER 2-3[1]

P. W. Whiting, *Local Secretary*
University of Pennsylvania, Philadelphia, Pa.

Bedichek, Sarah, North Texas Agricultural College, Arlington, Texas: *Sex balance in the progeny of triploid Drosophila.*—Males from eight X-IV translocation stocks were crossed to triploids with recessive markers, producing sterile intersexual "duplication intersexes" with a short fragment of the X plus two complete X's and three autosomes and weakly fertile hypotriploid females with longer X fragments plus 2X3A. Duplication intersexes bearing interior regions of the X in triplicate were studied by combining right and left hand fragments from two different translocations. Of eight duplication intersexes with very short sections, covering successively the entire X chromosome, in triplicate, none showed a marked shift in the female direction. Therefore a single primary sex gene can not exist capable of producing a functional female when represented three times with 3A regardless of the dosage of the remainder of the X chromosome. There was a graded shift toward femaleness in the phenotypic appearance of individuals with an X fragment plus 2X3A with increasingly longer fragments. Individuals were hypotriploid with either right or left hand section of two translocations (t-lz and lz-v breaks). Furthermore, fertility of certain fragment plus 2X3A aneuploids begins before the last trace of intersexuality vanishes, since some hypotriploids with the right hand fragment of the t-lz and lz-v breaks possess very rudimentary sex combs. For this reason and the fact that shorter sections from the left than from the right hand region of the X chromosome plus 2X3A result in weakly functional females, we may conclude that the portion of the X to the left of the t-lz break seem a little more female potent in relation to its cytological length than the portion to the right of this break. A drop in temperature to 18° C. produces intersexuality in those individuals carrying the right hand fragment of the t-lz break but does not alter the female appearance and function of hypotriploids bearing longer fragments. These results make more plausible the multiple sex gene theory of Dobzhansky and Schultz.

Blakeslee, A. F. and Avery, A. G., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Induction of diploids from haploids by colchicine treatment.* — By spraying growing points of haploids of *Datura stramonium* with colchicine solutions, doubling of chromosome number has been brought about with abundant production of 2n flowers and 2n capsules resulting. Since there is only one chromosome of a kind in haploid Daturas, doubling the chromosome number gives rise to diploid races which are homozygous except for possible new mutations which have not been observed in tests of ten induced 2n capsules from treated haploids.

If methods for the induction of haploids from diploids which are being investigated are successful, a ready means would be available for securing homozygous 2n races from highly heterozygous material (such as species hybrids) by doubling the chromosome number of such induced haploids.

Boche, R. D., Carnegie Institution of Washington, Baltimore, Md.: *Hymenopteran parasitism of Drosophila.—Eucoila drosophilae* Kieffer (Figitidae) is a small hymenopteran which parasitizes various species of Drosophila. Oviposition is made into the young larvae of the first or second instar. The parasite egg throws off a gelatinous membrane within two minutes after being laid. This membrane persists during embryonic development, and its method of formation appears to be different from any known to the author among insects.

The larva hatches out of the enclosing membrane in two days, and lies free in the body cavity of the host larva. The host continues its development normally through pupation and histolysis; development of the imaginal discs is well underway when the parasite which has now become a grub begins the active destruction of tissue. After consuming the contents, the grub passes through a metamorphosis within the pupa case and emerges as an adult wasp. The total period of development thus occupies 18 to 20 days. The adult lives two weeks, hence the generations do not overlap.

Not all species of Drosophila are suitable hosts for the parasite's development, some being absolutely immune. Susceptible and immune forms are found among species both closely and distantly related to *D. melanogaster,* which is itself completely susceptible. Within *D. melanogaster,* moreover, no immune strains could be found among numerous wild type and mutant forms tested.

[1] Printed by courtesy of The Collecting Net. Definitive publication will follow in *Genetics.* Reprints of abstracts can be obtained if ordered from The Collecting Net at the time of the meeting.

Evidence from the distribution of eggs among host larvae shows that oviposition is certainly not a random one and suggests that the female parasite is able to discriminate between parasitized and unparasitized hosts.

Buchholz, J. T., University of Illinois, Urbana, Ill., and Blakeslee, A. F., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Direct demonstration in the male gametophyte of the result of crossing-over in Datura.*—The gene sl-2 has been located in the 5·6 chromosome. It slows pollen-tube growth to nearly half the normal rate, but is nevertheless pollen transmissible through splicing of styles, so that homozygotes in the sporophyte generation were obtained. Two pollen abortion genes (pa-2 and pa-12) are also located in the 5·6 chromosome. It was therefore possible to combine these pa genes (using the latter as females) into plants with formulas sl-2/pa-2, and sl-2/pa-12. The first yields gametophytes pa-2, sl-2 from non-crossovers and (sl-2 pa-2) and (++) from crossovers. All pollen grains with pa-2 abort so that only two classes, sl-2 and (++) are viable. Preparations of pollen tube test-slides show a large group of short sl-2 pollen tubes and a small group of (++) tubes (crossovers) which grow out as long as normal pollen tubes. The preparations show that the crossover value for sl-2/pa-2 is very low (± 1%), that for sl-2/pa-12 is much higher. Plants of the formula sl-2/pa-2 yield so few (++) pollen tubes that they may be used as pollen parents for the rearing of sl-2 homozygotes or for any pollination in which it is desired to transmit this gene through the pollen. If these plants are used as females they give offspring that are carriers of pa-2 if half the pollen is aborted, and carriers of sl-2 if the pollen is good.

Buck, John B., Carnegie Institution of Washington, Baltimore, Md.: *Structure of living salivary gland chromosomes.*—Since the usual method of examining "living" salivary chromosomes by dissection under paraffin oil was found to be very unreliable, recourse was had to a technique by which the chromosomes were studied directly through the body wall of the living larva. From an examination of the larvae of Sciara, Drosophila, Simulium and Chironomus, the following tentative conclusions were drawn concerning the visible structure of normal living salivary gland chromosomes:

(1) In all the species studied there is very little free fluid in the nucleus, i.e., the chromosomes (plus nucleolus and chromocenter, where present) fill, or nearly fill the nucleus.

(2) The banding in Sciara and Drosophila is sparse and indistinct, that in Simulium and Chironomus sharp, detailed and in some respects like that in fixed preparations.

(3) In all of the forms most of the bands are either smooth and uniform or finely granular. Few or no vesiculated bands are seen.

(4) In optical cross-section, the chromosomes are round and have a dark margin and uniformly granular interior.

(5) No longitudinal fibrillae ("striations," "chromonemata"), such as appear in aceto-carmine preparations, are visible.

(6) No pronounced foam or alveolar structure of the achromatic regions is visible.

Burks, Barbara S., Carnegie Institution of Washington: *A case of autosomal linkage in man: congenital tooth deficiency and hair color.*—The first clear case of autosomal linkage in man has been established through data in the family schedules of the Eugenics Record Office, and further corroborated through clinical study of families located in the field.

The "single-generation" method, or method of like and unlike sibling pairs, was used for detecting linkage between hair color and congenital tooth deficiency. Although the mode of inheritance of neither trait had been clearly defined up to now, it was possible to use the linkage relationships between them to arrive at a reasonable hypothesis as to the genetic transmission of both traits. It was also possible to estimate their recombination ratio (approximately 14 per cent), and to establish the genetic equivalence of missing third molars and other missing teeth, which had hitherto been thought to be independent traits.

Carothers, E. Eleanor, State University of Iowa, Iowa City, Ia.: *A Hybrid Acridian Gynandromorph.*—A species cross, *Trimerotropis citrina* ♂ × *T. maritima* ♀, produced among more than 100 normal F_1 males and females one nearly perfect bilateral gynandromorph. Externally the left side was male and androgenetic; the right, female and a typical F_1 blend of the characteristics of the two species. The right halves of the ovipositor valves were fully developed and were opened and closed normally as maturity approached. The gynandromorph attracted males and also attempted to court females. Apparently, copulation was impossible structurally. Internally there was a right ovary with fully developed eggs and a normal right oviduct. No trace of either testis or ovary was found on the left side. Any explanation of the mechanism involved will have to take into consideration the following facts. 1. This is a half and half gynandromorph; therefore, whatever the mechanism, it became operative at the first cleavage. 2. The male half is androgenetic at least in regard to all pigment and pattern factors. 3. The female half is biparental.

4. The pigment and pattern factors are non-sex-linked as shown by reciprocal hybrids; therefore, the X chromosome in the male half could have come from either parent. 5. Parthenogentically produced acridines restore the diploid number of chromosomes by means of an extra prophase split without cleavage (King and Slifer '34) and are always females. If this gynandromorph was produced by chromosomal elimination one or more autosomes as well as an X were involved. It is highly questionable if such a cell would live and cleave. If dispermy is the explanation, and the diploid number was restored as in parthenogenesis, one X must have been eliminated or else the original one did not double when the autosomes did.

DOBZHANSKY, TH., and KOLLER, P. CH., California Institute of Technology, Pasadena, Cal., and Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Sexual isolation between two species of Drosophila—a study on the origin of an isolating mechanism.*—Chiefly intraspecific, and only few interspecific, matings occur in mixed cultures containing equal numbers of females of *D. pseudoobscura* and *D. miranda* and of males of one of those species. After a 5 days exposure, approximately 95% of the females of the species to which the males belong, and less than 10% of the females of the other species, are impregnated. After a 21 days exposure, less than a quarter of the foreign females are fertilized.—The two races of *D. miranda,* coming from the Puget Sound region and from Mount Whitney respectively, show a preference for homogamic matings; this preference is much less pronounced than that observed in mixed cultures of *D. miranda* and *D. pseudoobscura.*—In cultures containing females of only one species and males of the other a varying proportion of the females, but not more than 51%, is fertilized after a 9 days exposure. If the *D. miranda* females used in such experiments belong to the Puget Sound race, the proportion fertilized depends upon the strain of *D. pseudoobscura* furnishing the males. With a single exception, strains of *D. pseudoobscura* from localities lying in or near to the area inhabited by the Puget Sound race of *D. miranda* give a lower frequency of fertilization than do strains coming from remote localities. No such result is obtained in experiments where the Whitney race of *D. miranda* is used. Thus strains of *D. pseudoobscura* show not only genetic differences in their reactions toward *D. miranda,* but these reactions are not identical toward the two races of the latter species. It is concluded that genetic variability affecting the behavior of the flies in interspecific crosses is present within either of the two species studied. Such variability may furnish building blocks from which isolating mechanisms can develop.

DOVE, W. FRANKLIN, University of Maine, Orono, Me.: *A study of individual versus group needs as a theoretical and an experimental approach to aggridascendance.*—From statistical analysis of the nutritional needs of all individuals of the group, the following observations have been made:

1. That preferences for foods are, in part, individual and, in part, group manifestiations of nutritional needs.

2. That when the group is treated as an ever-shifting population of rated individuals, the optimum *group* manifestations of nutritional needs (for growth) are similar to those of the *individuals* who have been rated during the period of growth to lie between the mean and the maximum for rate of growth. Since this position in the group array represents an ever-shifting series of different individuals, a *type* is indicated. And, since the type is integrated from individual (*id*) likeness-differences of all individuals of the group (*aggregate*), the superior or leader type has been called the *aggridant* type—an individual type integrated from the group as a whole.

3. That the aggridant type predetermines, specifically, which satisfactants, which ratios, and which quantities of satisfactants are necessary for group superiority. By following the desires of the aggridant, we have uncovered new food combinations which produce a growth rate as much as 30% above standard.

4. That the theory of aggridascendance is applicable also to the satisfaction of needs in general: to the planning of food-production programs, for instance, and to the analysis of needs inherent in the socioeconomic structure.

One tangible common denominator derived from these experiments is expressed by the aggridant in a specialization-diversification ratio. This S-D pattern of the aggridant type is one of the principal clues to a controlled phenogenesis—to aggridascendance.

EASTLICK, HERBERT L., University of Missouri, Columbia, Mo.: *A study of pigmentation in the chick embryo by means of limb bud transplantation.*—It has been demonstrated by means of limb bud transplants made between 40 to 70 hour chick embryos that cells lying adjacent to the neural tube (presumably neural crest cells) are responsible for pigmentation of the fowl.

1. Grafts made between pigmented donors and pigmented hosts of the same age illustrate this clearly, providing the grafts become attached to the mesenteries of the host. If the right limb bud of the donor is cut free at the edge of the

neural tube and the left limb bud of the same donor is severed at the level of the intermediate cell mass, the former transplant develops pigmented plumage while the latter possesses white feathers.

2. Brown Leghorn grafts made to White Leghorn hosts develop colored plumage if the pigment forming cells are included but in the absence of such cells white plumage results.

3. White Leghorn grafts made to pigmented hosts show that all transplants which become attached to the mesenteries develop typical White Leghorn plumage; transplants attached to the body wall by a narrow bridge are intergrades, while well attached grafts are always intensely pigmented.

Pigmentation of these grafts seemingly is due to the migration of neural crest cells from the host. The pigment forming cells apparently do not cross the mesenteries. If pigmentation were due to a diffusible substance, the grafts attached to the mesenteries should become colored.

The results indicate that the inhibitor in the White Leghorn acts specifically within the pigment forming cell. The inhibitor apparently is unable to block pigment formation by foreign neural crest cells.

Howard, Alma, McGill University, Montreal, Canada.: *Chromosome behaviour in relation to cancer susceptibility in mice.*—The discovery by Huskins and Hearne that a correlation exists in inbred mouse lines between high frequency of mammary gland cancer in females and low frequency of chiasmata in the primary spermatocytes of males has been confirmed and substantiated by further work on the same and other strains. Chiasma counts on hybrids between high and low chiasma frequency lines have shown, however, that unlike cancer susceptibility as reported by Little and others, chiasma frequency is not subject to "maternal inheritance." Nevertheless it is influenced to some extent by the milk which the young mouse receives, and in this way its behaviour resembles that of cancer susceptibility as reported by Bittner.

Hughes, Roscoe D., Medical College of Virginia, Richmond, Va.: *The chromosomes in the hybrid between Drosophila virilis virilis and Drosophila virilis americana Spencer.*—A study of the salivary gland chromosomes of the hybrid between *D. virilis virilis* and *D. virilis americana* reveals striking differences in the linear arrangement of the bands in the two sub-species. If we use *D. virilis virilis* as the standard for comparison, then the hybrid shows that there are major inversions in chromosomes 1, 2, 4, and 5. Chromosomes 3 and 6 are essentially similar. Pairing is typically loose except in chromosome 3. There is no evidence for interchange between non-homologous arms. There appear to be no large blocks of bands in either sub-species which do not have homologous regions in the other sub-species. Metaphase plates of larval ganglia cells also show striking differences. Both the male and the female of *D. virilis virilis* have five pairs of rod-shaped chromosomes and one pair of dots. In the *D. virilis americana* female there are two pairs of V-shaped chromosomes, one pair of rods, and one pair of dots. The male of this sub-species has three V-shaped chromosomes, four rods, and one pair of dots. Two of the rods in the male appear to form a pair corresponding to the one pair of rods in the female. The other two rods seem to pair with one of the V-shaped chromosomes, one with each arm. I am indebted to Dr. Warren P. Spencer for supplying the stocks of *D. virilis americana.*

Jollos, Victor, Madison, Wis.: *Further tests of the role of cosmic radiation in the production of mutations.*—The mutation rate of two pure inbred stocks of *Drosophila melanogaster* was tested following exposures to various frequencies of cosmic radiation. Exposures at an altitude of 14000 feet under 18 mm. lead produced an increase in the mutation rate about 3.32 times that at Madison altitude without lead. Exposures at the high altitude without lead, and at Madison under lead, increased the mutation rate about 1.36 and 1.30 times respectively. The results are consistent in both stocks and in separate periods of exposure. But the increase in the mutation rate is statistically significant only following exposures at the high altitude under lead.

The frequencies of cosmic radiations in the exposures are estimated to be 15, 5-6 and 3 times respectively higher than those at Madison without lead.

The figures for the exposures at Madison without lead and at the high altitude under lead indicate that not more than 16.67% of the "spontaneous" mutations at Madison altitude could be related to the influence of cosmic radiation. A general formula of the quantitative relation between intensities of cosmic radiation and frequencies of mutation can be derived, under the assumption that the mutation rate increases in a straight line, in proportion to the increase in cosmic radiation frequencies.

The results of the other exposures agree with this assumption. But they are not yet conclusive.

The tests and calculations permit only a rough first approach to a determination of the quantitative relation between cosmic radiation intensities and frequencies of mutations. The basic figures in the calculations are not yet sufficiently reliable,

either on the physical or on the biological side. Necessary improvements in the measurements are discussed.

JONES, DONALD F., Connecticut Agricultural Experiment Station, New Haven, Conn.: *Variable effect of the C locus in maize following translocation.*—The paired dark and colorless areas in the aleurone layer of the corn kernel are known to be the result of segmental shift. The usual result of increasing the number of C alleles is an increase in color whether this is brought about by germ cell recombination or somatic cell recombination. In a few cases it has been noted that a chromosomal shift in the C region produces a color lighter than normal instead of darker. Clear cases have been found of colorless areas paired with areas lighter than the surrounding normal cells. In a few cases of inter-chromosomal exchanges, involving both C and Pr, light areas adjoin colorless and red areas. Light areas may also adjoin colorless and dark areas. Evidently the relocated C region not only fails to function as usual but prevents the normal action of the other C allele. In this way it acts like a partial inhibitor of color. This is especially significant in view of the fact that one of the known alleles at or near the C locus is an inhibitor of aleurone color that is variable in its action in different lines.

KALISS, NATHAN, Columbia University, New York, N. Y.: *The expression of a short lethal deficiency in Drosophila melanogaster.*—Male *D. melanogaster* embryos, hemizygous for an X-chromosome with the terminal deficiency *260-2,* develop to fully formed larvae which fail to hatch from the egg. Deficiency *260-2* (Demerec, D.I.S. 7:39) has at least four known loci missing: *chlorotic, lethal-JI, yellow,* and *achaete.* Live mutants, in contrast with normal animals of comparable age, are characterized by the feebleness of their hatching movements, by the large accumulation of calcium carbonate concretions in the proximal two-thirds of the anterior pair of the Malphighian tubules, by the irregular distribution of gas in the tracheal system, and by the inability of the animals to withstand dehydration. The pigmentation of their mouth armature and cuticular spines is "yellow-type" in color, in contrast to the darker pigmentation of wild-type animals of comparable age.

The mutants die within the egg membranes about 48 hours after oviposition (at 25° C.). When placed in an isotonic or hypotonic medium they may survive up to about 80 hours without further development. The increase in length of life is probably due to the prevention of dehydration which the mutants undergo on the usual food medium. Their histomorphology is indistinguishable from normals of comparable age.

Death of the mutants can not be ascribed to the pathological condition of any specific organ. It must be ascribed to some physiological upset which is operative at about hatching time, since the development and differentiation of the mutant embryo proceeds normally up to this time.

KAUFMANN, B. P., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *An X-ray induced "reversed repeat."* — Among the F_1 progeny of a male *Drosophila melanogaster* irradiated at 3000r, one individual showed an intercalary duplication in the left limb of the third chromosome arranged in the pattern of a reversed repeat (abcd*gfeefg*hijk). Assuming that twin strands were present at the time of reunion of the broken sections resulting from irradiation, origin of the duplication may be attributed to fusion of sister chromatids at the same level, thus,

abcd | efg | hijk·
abcd | efg hijk·

giving abcd*gfeefg*hijk·, and probably another strand of the constitution abcdhijk·, whose fate remains conjectural. The union of sister chromatids in the manner here indicated offers a possible explanation of the origin in nature of such reversed repeats as occur in the chromosomes of Drosophila.

KIMBALL, RICHARD F., Johns Hopkins University, Baltimore, Md.: *A lag in the change of phenotype following a change of genotype in Paramecium aurelia.*—In the ciliate protozoan, *Paramecium aurelia,* the mating type is known to change in definite ratios at endomixis. When a change occurred in genotype from Type II to Type I, some of the animals remained phenotypically Type II for a while as shown by the fact that animals of both types were found for a short time after endomixis. However, all the animals later became Type I whether they were desecended from animals that, shortly after endomixis, were Type I or Type II. Therefore, all the animals in question were genetically Type I. It appears, then, that the presence of Type II individuals for a time after endomixis was due to a lag in the change of phenotype, not to a genetic difference. The rate of the change of phenotype differed both for different animals of the same line of descent and for different exendomictic lines. No lag was found for the change from Type I to Type II, possibly because it was too short to be detected. These phenomena are of interest from the point of view of the interaction of the genes and cytoplasm and of the time of action of the genes.

Koller, P. C., California Institute of Technology, Pasadena, Cal., and Carnegie Institute of Washington, Cold Spring Harbor, N. Y.: *Chromosome variation in populations of Drosophila pseudoobscura from contiguous localities.*—Samples of populations were collected in seven different canyons in the Panamint Mountains (California). The relative frequencies of various gene arrangements in the third chromosome were studied using the salivary gland technique. Populations inhabiting separate canyons were found to differ from each other, in spite of the fact that migration of flies from canyon to canyon is apparently not prevented by any natural barriers. It follows that even a population occupying a continuous habitable territory may become differentiated into local subgroups. It is suggested that this differentiation may be brought about by random variations in the frequency of the component types.

Landauer, Walter, Storrs Agricultural Experiment Station, Storrs, Conn.: *Types of cross-beak in fowl.*—1. Cross-beak as a common malformation of chicken embryos in association with eye defects; not hereditary; frequency influenced by environment. 2. Cross-beak in late homozygous Creeper embryos, more frequent in Japanese Bantams than in ordinary Creepers. 3. A hereditary form of cross-beak in which chicks are normal at hatching time, the beak becoming crossed between one and two months of age; not a single factor trait. 4. A hereditary form of cross-beak in which the chicks have a cross-beak at (and before) hatching, but of which a certain proportion develops a normal beak later; not a single factor trait. Crosses between birds with these two types of inherited cross-beak give exclusively normal offspring. 5. An inherited type of cross-beak in which the upper beak is short at hatching time, becoming crossed subsequently. No true-breeding individuals obtained of types 3 and 4, in spite of inbreeding. Many genetically cross-beaked birds probably have normal beaks. Embryonic mortality is not increased by the cross-beak trait (type 3), but post-hatching viability of chicks with cross-beak is much below normal. Common to all types of cross-beak (type 5 not studied) are structural abnormalities and asymmetries of the skull. The cross-beak is probably only a secondary symptom of these skull abnormalities. Among the non-hereditary cross-beak embryos with eye deformities crossing of the upper beak toward the left is much more frequent than the opposite type. The reverse is true for homozygous Creeper embryos and for the two types of hereditary cross-beak studied in this respect.

Loehwing, W. F., University of Iowa, Iowa City, Ia.: *Physiological factors involved in differentiation of male and female parts of the angiosperm flower.*—Recent work by the author and others shows flower formation to be due to a specific florigenic inductor (hormone), produced in leaves, which are the loci of photoperiodic perception, whence the florigen normally is transferred to adjacent buds causing inception of flower primordia therein. In some instances at least, the length of photoperiodic exposure requisite for functional megaspore formation is less than that for production of viable pollen. This fact underlies the frequent occurrence of pollen sterility of greenhouse plants in the winter. The usual peripheral position of staminate parts in the flower appears associated with higher oxidation potential at these points, due apparently to greater availability of oxygen and the stimulus of light. When light intensities are reduced, oxidation potentials in stamen loci fall and varying degrees of reversal in sex expression occur. Marked and highly localized nutritive differences distinguish regions of stamen and pistil formation. High sugar, phosphorus, phosphatase and oxidase characterize staminate and high nitrogen the pistillate zones. Data of the author indicate the importance of and necessity for micro-analysis on adjacent but often physiologically contrasted tissues. Normally there is no marked transfer of the flowering stimulus, but by certain combinations of exfloration and defoliation, evidence of transfer is demonstrable. Recent results suggest why earlier attempts to induce sex reversal by reciprocal grafts among staminate on pistillate plants of dioecious species were unsuccessful.

Margolis, Otto S., Western Reserve University, Cleveland, Ohio: *The effects of reduced atmospheric pressure and oxygen on facet number in bar-eyed Drosophila.*—Data on the effects of reduced atmospheric pressure and of a pure oxygen atmosphere on the development of facets in Bar-eyed Drosophila are presented.

Flies which had spent varying portions of their egg-larval period (2 to 5 days) at one-half atmosphere pressure showed a very small, but for the most part statistically significant decrease in facet number. Exposures to reduced pressure all took place before the beginning of the temperature effective period.

Flies developed from larvae which had spent from the 24th hour of development to the 72nd hour in a pure oxygen atmosphere showed a very marked increase in facet number over the control group which developed under normal atmospheric conditions at 28°. A second group which had spent from the 48th hour of development to the 90th hour showed a somewhat smaller but significant increase in facet number.

It is concluded from these results that facet determining processes are in operation before the beginning of the temperature-effective period, i.e., about 50 hours at 28°; and that these can best be studied through the systematic use of new and controllable environmental variables such as the ones used here.

MARSHAK, ALFRED, The New England Deaconess Hospital, Boston, Mass.: *The stage of mitosis at which chromosomes are rendered less sensitive to X-rays by ammonia.*—When cells irradiated with X-rays are examined at various intervals after irradiation and the per cent anaphases with unaltered chromosomes are plotted as a function of time after irradiation, a pronounced minimum is observed at three hours after irradiation. The cells in anaphase at three hours after irradiation were in the onset of prophase at the time of exposure to X-rays. It was previously postulated that the marked sensitivity at this stage may be attributed to the appearance of positively charged surfaces upon the division of the chromonemata at this time. The hypothesis is supported by the observation that ammonia greatly reduces the sensitivity of chromosomes to X-rays. The theory further required that the onset of the prophase be the stage which was principally protected by ammonia. To test this seedlings of *Allium cepa* were treated with 0.0025 N NH_4OH and given 80 roentgens of X-rays. Counts of the per cent normal anaphases from such seedlings showed a sharp maximum three hours after treatment in contradistinction to the minimum obtained from those treated only with X-rays. After five hours the curves obtained from both types of seedlings are identical, indicating that the sensitivity at the resting stage is not altered. These results, therefore, offer additional support to the above mentioned theory.

MATHER, KENNETH, John Innes Horticultural Institution, Merton, England: *Chiasma frequencies in trisomic maize.*—It has been shown previously that competition, or inter-chromosome intereference, as judged by the negative correlation of chiasma frequencies, is due to there being an upper limit to the number of chiasmata which may be formed in a nucleus. The different bivalents compete for these chiasmata. Certain further considerations, notably the behaviour of tetraploids, indicate that this upper limit is related to the number of chromosomes present. The more chromosomes in the nucleus, the higher is the limit to the number of chiasmata which may be formed. On this basis, the addition of an extra chromosome to a diploid showing competition should lead to (a) a decrease in the strength of the competition and (b) a corresponding increase in the mean number of chiasmata formed in the nucleus, because the extra chromosome will raise the limit without increase in the length paired at pachytene, i.e., the length of chromosome effective for chiasma formation. A study of maize trisomics, made available by the kindness of Drs. Rhoades and Anderson, has fully verified these conclusions. Five plants carrying an extra short arm of chromosome five show a definite decrease in the negative correlation as compared with five sister diploids, and also have a correspondingly increased frequency of chiasma formation. Less extensive data on other trisomic strains are in agreement with these results. We may, then, relate the upper limit to the number of chiasmata in the nucleus to the number of chromosomes present.

METZ, C. W., Department of Embryology, Carnegie Institution of Washington, Baltimore, Md.: *Structure of salivary gland chromosomes in Diptera.*—With accumulated evidence showing that a salivary gland chromosome is "solid", not a hollow cylinder, earlier views considering it made of spirally disposed chromonemata or "genonemes" around a central core have largely been abandoned. Observers still maintain, however, that numerous chromonemata *are visible* in these chromosomes. Bauer (on Chironomus) and Painter and Griffen (on Simulium) give detailed accounts based on this view. It should be pointed out that these accounts do not agree as to the nature of what are called chromonemata. In the writer's opinion, based on study of both these genera as well as Sciara and Drosophila, the so-called "chromonemata" here are different aspects of an essentially honeycomb or alveolar organization. (Whether there is an underlying *invisible* organization made up of chromonemata is another question.)

Evidence will be presented on this subject. Space merely permits mentioning a few points here. E.g., according to our evidence "chromonemata" in Chironomus (1) form diagonal crisscross at all optical levels in side view, (2) regularly intersect at granules, (3) are often continuous diagonally across *both* chromosomes of a pair. These are properties of honeycomb, not discrete, sister threads confined to one chromosome. Simulium "chromonemata" interpretation apparently (1) is based on assumption considering as unit "chromomeres" droplets apparently made of material from two bands, one of which may be removed by deficiency, (2) disregards granules and "chromomeres" lying between the "chromonemata", (3) gives special significance to products of distortion.

METZ, C. W., Department of Embryology, Carnegie Institution of Washington, Baltimore, Md.: *Observations on the nature and significance of*

minute chromosomal differences in species hybrids and in ordinary wild strains of Sciara.—Dobzhansky and Tan have noted two types of chromosome differences in salivary gland chromosomes in Drosophila species hybrids—large ones due to gross chromosome rearrangements (mainly inversions and translocations), and minute ones involving only one or a very few "bands". In Drosophila the former type appears to be relatively common and the latter rare, both in hybrids and within a species. In Sciara, however, the situation appears to be reversed. Extensive study of salivary gland chromosomes in three species has revealed no case of inversion and only two of translocation, including both irradiated and non-irradiated material. Likewise, none has yet been definitely identified in the species hybrids. Minute differences, however, are common, both in pure species and hybrids. Ordinary mutation rate, with or without irradiation, is relatively high in Drosophila and very low in Sciara. It seems probable that lack of mutation in Sciara is due to lack of inversions and translocations, and that this latter is in turn due to some general difference in chromosome constitution or behavior in the two genera.

This supports the view that most "mutations" are mechanical chromosome rearrangements. Characteristics of the minute chromosome differences lead to the hypothesis that small (mostly single band?) duplications, followed by qualitative gene changes provide a primary mechanism of evolution in Sciara.

Metz, C. W., Department of Embryology, Carnegie Institution of Washington, Baltimore, Md.: *A recurrent, spontaneous break in a salivary gland chromosome in Sciara.*—In one particular wild-type stock of *Sciara ocellaris,* numerous individuals exhibit in salivary gland nuclei a complete, transverse break in one member of chromosome pair *A,* at a definite locus about two-fifths the distance from end *l* (possibly the spindle fiber locus). A small deficiency may be involved. Break appears to be present in all salivary gland cells of an affected individual, but no broken chromosome has been found in any of the numerous mitotic figures of ovarian cells examined in these individuals.

Apparently the break occurs in salivary gland cells during ontogeny, in a regular manner. No case has been found in which both homologues are broken. The phenomenon or condition appears to be inherited, and preliminary data suggest that half the offspring from a pair mating show the effect. No such condition has been found in other stocks.

The case may have bearing on problem of the mechanism of chromosome rearrangements. On the hypothesis that chromosome breaks precede rearrangements and that free, broken ends attract one another, it might be expected that union of the closely approximated free ends would occur here, but apparently such is not the case. It seems probable that the break is not mechanical, but is "autonomous", and that it occurs early in ontogeny. Conditions in other glands, etc., are being investigated.

Payne, Nellie M., University of Minnesota, Minneapolis, Minn.: *The "yellow" colors in Microbracon hebetor (Say) [Habrobracon juglandis (Ives.)].*—There are at least three chemical groups of "yellow" colors in the adults of *Microbracon hebetor.* By "yellow" is meant any color in the range from white to red provided there is at least one "yellow" in the series. The series of alleles called "carrot" belong to the carotinoids. These differ in intensity according to the degree of oxidation. A second series of eye colors appears to belong to the flavone group. The body "yellow" is not closely related chemically to either of the eye colors. The black which occurs in the wild-type wasps is related to the proteins. In the wild-type, "yellows" may occur in the eyes and body but their presence is obscured by the melanin.

Poulson, D. F., Yale University, New Haven, Conn.: *Polyploidy in eggs of Drosophila melanogaster carrying X-chromosome deficiencies.* — In zygotes deficient for the entire X-chromosome (nullo-X) development becomes abnormal through the failure of the regular distribution of nuclei to the surface and the subsequent failure of blastoderm formation. In eggs deficient for either the left or the right halves of the X-chromosome normal development ceases at slightly later stages. Of the cytological abnormalities common to these three deficient types, the most frequent and conspicuous are polyploid nuclei and multinucleate cells. These are usually found in nests and may lie in any region of the egg occupied by nuclei or cells. The nuclei may be tetraploid or of a higher degree of polyploidy. While the multinucleate cells most probably arise when cell division fails to keep step with nuclear division, there are two likely ways of obtaining the polyploid nuclei. The chromosomes may divide without the rest of the cell dividing and remain in the same nucleus; or, the nuclei may divide without the cell dividing and in the next division the spindles of the resulting multinucleate cell may fuse to give polyploid nuclei. The latter is very frequent in these eggs. Incomplete fusion of spindles may give multipolar spindles and abnormal distributions of chromosomes. If there is sufficient space the spindles do not interfere, and the number of nuclei increases. The behavior of the spindles is much the same in syncytial regions

of the eggs where cell formation fails or is incomplete. The situation reported is very similar in many respects to that found in the testes of A/B hybrids of *D. pseudoobscura* (Dobzhansky).

RAFFEL, DANIEL: *A genetic analysis of apparent losses of the distal end of the scute 8 chromosome.*—When males containing the scute 8 chromosome, which contains an inversion the left break of which occurred between the loci of achaete and scute and the right break beyond bobbed and Block A in the chromocentral region, are irradiated, many cases of simultaneous yellow, achaete, and lethal mutations arise as was found by Levit, Muller, Patterson and others. These cases were at first interpreted as losses of the distal ends of these chromosomes extending to the original point of breakage or extending to some point located in the chromocentral region that had been brought just to the right of the achaete locus. Investigation of several of these supposedly truncated X chromosomes by Prokofyeva showed that these left ends were present but indicated that minute rearrangements had occurred in them. The author carried out a genetic investigation to determine whether in all such cases the locus of lethal J1, the only lethal to the left of yellow of which a stock exists, was either lacking or affected. Males carrying lethal J1 covered by the left end of scute J4 (a translocation to the third chromosome—in these flies the crossover suppressor CXF and Dichaete were present in the homologous third chromosome) were mated with females of 15 stocks containing yellow, achaete, and lethal mutations obtained by Belgovsky by irradiating sc^8 B W^a X chromosomes. In five of these fifteen crosses females with Bar and Dichaete were obtained. Here scute J4 was absent and lethal J1 must have been "covered" by the X chromosome containing the yellow, achaete and lethal mutations. This demonstrates that in these five cases, at least, the simultaneous mutation of the yellow, achaete, and a lethal was not due to the loss of the left end of these chromosomes. It shows also that other lethal genes besides lethal J1 lie to the left of achaete. And this, along with Prokofyeva's cytological investigations, removes the last evidence for the survival of so-called simple breaks or the possibility of the existence of chromosomes lacking the terminal piece. Hence it supports the conception of the telomere or terminal gene as a permanent chromosome structure.

REED, S. C., McGill University, Montreal, Canada: *Determination of hair pigments in the mouse.*—Transplantation techniques have provided information regarding the time of determination of the coat pattern "black-and-tan." This method has also been used to indicate the presence of some substance which will cause "non-waved" cells to produce "waved" hair. A further modification involving the use of the chorio-allantoic graft has shown that the pigment inhibitor in White Leghorn fowls is either specific to White Leghorns or is not present in the allantoic blood stream.

SAX, KARL, Harvard University, Cambridge, Mass.: *The effect of X-rays on chromosome structure.*—An analysis of irradiated microspores of Tradescantia has led to the following conclusions. (1) A single X-ray "hit" can break two adjacent sister chromatids. (2) Broken ends of sister chromatids invariably fuse to form a bridge and a U-shaped fragment at anaphase. No such fusion occurs if the break occurs in the resting stage before the chromosome is split. (3) Induced breaks do not occur at random in the chromosome, a fact which indicates that secondary factors play some part in breakage. (4) There is no temperature coefficient for X-ray-induced aberrations. (5) The chromosomes are most susceptible to X-rays during prophase, and are more suceptible to breakage at meiosis than at mitosis. The differential susceptibility is attributed to mechanical factors. (6) Simple breaks are produced by single hits, but fusions between different chromosomes are caused by two independent hits. The relation between dosage and chromosome aberrations approaches a two hit curve,—breaks $= 1-e^{an}(1-an)$. The discrepancy between this curve and the mutation curve $(1-e^{an})$ can be attributed to the fact that most of the visible chromosome aberrations are of the type which would not survive many cell generations. (7) Mutations produced by mechanical changes in the chromosome, induced by a single "hit", can be attributed to breaks in adjacent gyres of the chromonema leading to small inversions and deficiencies. (8) Few of the X-ray "hits" on the visible chromonema result in either chromosome aberrations or mutations.

SCHWEITZER, MORTON D., Cornell University Medical College and the New York Hospital, New York, N. Y.: *Role of heredity in childhood rheumatism.*—(This report is based on family studies made in the clinic of Dr. May G. Wilson and was aided by a grant from the Commonwealth Fund.)

Making use of techniques for the application of genetic analyses to the features of human clinical material, the role of heredity was unequivocably verified. Confirmatory were the results with twins. Four pairs of identical twins were all affected, while among 12 fraternal pairs, 7 showed the disease in only one member.

Factorial analysis showed agreement with a single recessive gene hypothesis, the standard error being very small. Simple dominance, sex

linkage, two or more recessive or dominant gene hypotheses were excluded. Comparable analyses of data from other laboratories gave concordant results. The penetrance (expression) of the disease in this material was quite high, (82%) thus facilitating the analysis without the need for introducing statistical corrections.

The varying incidence in different communities and the marked paucity of cases among the children of people in favorable economic circumstances as compared with the usual clinic population is interpreted to mean that the presence of an hereditary susceptibility will not necessarily predestine every bearer to fall prey to childhood rheumatism. It is believed, rather, that definite environmental factors, general or specific are also required. This is consistent with the judgment of clinicians.

The verification of heredity as a dominant factor in the etiology of childhood rheumatism will make possible a reinvestigation of the various hypotheses regarding etiological agencies which have hitherto given equivocal results. It also provides a means of evaluating the efficacy of proposed preventive measures. Within a family where the disease occurs, it is now possible to assay the likelihood that a given child may fall victim to childhood rheumatism.

Singleton, W. Ralph, Connecticut Agricultural Experiment Station, New Haven, Conn., and University of Missouri, Columbia, Mo.: *Cytological observations on deficiencies produced by treating maize pollen with ultra violet light.*—A recessive stock lg_1 gl_2 b_1 v_4 was pollinated with dominant pollen rayed from 2-8 minutes with ultra-violet using a Hg 90 filter. Of 1248 seedlings five showed recessive characters and were studied cytologically. Three (lg gl b) were haploids. One (v_4) had a heterozygous deficiency of 80% of the long arm of chromosome 2. The virescent character was accentuated over the normal condition. One was recessive for *b* initially but later developed fine streaks of sun red (possibly B). Progenies of this plant are being studied further.

In another experiment plants recessive for *a* were pollinated by dominant pollen rayed from 4-8 minutes using a Hg 90 filter. Of 2289 seedlings, 12 green ones (a) were sampled cytologically. Two were haploids. Three showed deficiencies for portions of the long arm of chromosome 3. One showed no deficiency.

In a third experiment pollen of the constitution *ABPl* was treated for 16 minutes with the discharge tube giving nearly monochromatic radiation of 2537 Ångström units. Sporocyte material was collected from 162 plants (the whole population) and stored in a refrigerator until pollen samples were examined. Plants showing segregating pollen were then examined cytologically. Of the 162 plants, 31, or 19%, had segregating pollen. Diakinesis figures showed 10 pairs for all. One brown plant, *aBPl*, was deficient for 60% of the long arm of chromosome 3. Two plants showed no deficiency. One plant was deficient for the short arm of chromosome 3. One had two deficiencies on chromosomes 4 and 8.

All heterozygous deficiencies so far observed are apparently terminal. No translocations were found.

Warmke, H. E., and Blakeslee, A. F., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Induction of tetraploidy in Nicotiana sanderae and in the sterile hybrid N. tabacum × N. glutinosa by colchicine treatment.*—From a series of plants of *N. sanderae* the seeds of which had been treated with different concentrations of colchicine (.2 to .8%), 35 have already flowered. Of these 18 (51%) have been found to be wholly or partially tetraploid as determined by pollen size. Seeds of the tetraploid flowers are distinctly larger than those from diploids. 4n corolla tubes are greatly enlarged. The second generation 2n and 4n offspring have been obtained and are being studied cytologically.

Seeds from the cross *N. tabacum* × *N. glutinosa* were supplied us by Dr. F. O. Holmes. These hybrids were all sterile with ± 98% aborted pollen grains. Growing points of the hybrids were sprayed with 1% colchicine in a lanolin emulsion, which has been found more effective than spraying with solutions. Characteristic roughening of leaves resulted in all cases. Of 39 plants, five (13%) have produced branches with different amounts (10 to 90%) of good pollen, and three have already set capsules with seeds following hand selfing. By colchicine treatment the chromosome number has been doubled, allowing the chromosomes of the parental species to pair *intra se*. The sterile hybrid has been transformed into a fertile allotetraploid.

Weinstein, Alexander, Zoological Laboratory, Columbia University, New York, N. Y.: *The arrangement of the chromatids in crossing over.*—The arrangement of the chromatids in the tetrad depends on the number of levels of crossing over, the distances between them, the number and nature of the exchanges at each level and their relation to exchanges at other levels (recurrence). These points have been tested by an analysis of all available data in *Drosophila melanogaster* and *D. virilis*.

Crossing over between sister chromatids is excluded because it would lead to negative frequencies of tetrads and, in attached X's, to values of homozygosis that are too low. Crossing over between more than two chromatids at a level would lower or eliminate homozygosis in attached

X's. Recurrence must be random for the following reasons. (1) An excess of regressives over digressives would involve negative frequencies of tetrads. (2) An excess of digressives over regressives would lead to coincidence values greater than 1 and to tetrad frequencies that are irregularly distributed or negative. (3) An excess of progressives would produce in attached X's too high a proportion of homozygosis and too low a proportion of individuals completely heterozygous; a deficiency of progressives would lead to deviations in the opposite directions.

These results make it possible to calculate in the tetrad the frequencies of internodes of various lengths and to determine on a genetic basis how the chromatids are arranged at and between levels of crossing over.

White, M. J. D., University College, London, and Columbia University, New York: *A new type of anomalous meiosis.*—The general course of meiosis is remarkably similar for all organisms, the only animals with really anomalous meiosis falling into three groups; (1) animals with male haploidy, (2) some Scale Insects, (3) the male sex in many of the Diptera. Up till now the absence of crossing over in the male Drosophila and Sciara has been unparalleled elsewhere, the only other animals in which crossing-over is entirely suppressed in one sex being those with haploid males. The present paper deals with the meiosis of some Mantids (Orthoptera) in which no chiasmata are formed in the male, and in which crossing over is presumably absent in that sex. The species dealt with are two: *Callimantis antillarum* Saussure (from the island of Haiti) and *Acontiothespis* sp. (from Yucatan). The first has eight pairs of autosomes, the second has only seven; both species are XO in the male. The usual diplotene and diakinesis stages are entirely absent in the male, and the first meiotic division is reductional for all parts of all chromosomes. Unfortunately the female meiosis is unknown up to now. Since in these Mantids there is no somatic pairing of homologous chromosomes, as in Drosophila, the existence of meiosis without formation of chiasmata raises some important points in connection with modern theories of meiosis.

Whiting, P. W., The University of Pennsylvania, Philadelphia, Pa.: *Sex-determination and reproductive economy in Habrobracon.*—Theory of sex-determination proposed in 1933 was proved by sex-linkage for inbred stocks as shown in females (Whiting: Amer. Nat. 68:68) and in males (Bostian: Amer. Nat. 69:57-58). In outcrosses sex-linkage is not obvious (Dordick: Genetics 22:191) and biparental males are replaced by females thus increasing reproductive economy in the species (Whiting: J. Hered. 26:263-278). Various theories have been suggested to explain reproductive economy and failure of sex-linkage in outcrosses: differential maturation and chromosome breakage (Whiting), multiple factors (Snell: PNAS 21:446-453). At present a theory of multiple alleles appears best to fit the outcross pedigrees. None of these theories contradicts the principles of sex-determination as established for inbred stock.

Woolley, George W., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me., and Cole, Leon J., University of Wisconsin, Madison, Wis.: *A New Dwarf Mutation (dw_2) in Rattus norvegicus.*—A second dwarf character in the rat differs in many respects (time of appearance, type of growth, reproductive ability, etc.) from the dwarf rat described by Lambert. Like the first described dwarf its expression depends upon the action of a monogenic recessive mutation.

The character may prove of value to the endocrine as well as the genetic field.

The mutation was first noted by one of us (Woolley) in the genetics colony at the University of Wisconsin where study was started. Recently the mutation has been transferred to the Roscoe B. Jackson Memorial Laboratory for further study.

STUDIES ON FRAGMENTS OF CENTRIFUGED NEREIS EGGS

Dr. D. P. Costello
Assistant Professor of Zoology, University of North Carolina

The unfertilized ovocyte of Nereis is somewhat flattened in a polar direction, measuring about 140 microns in diameter when viewed from the pole, and about 100 microns high in side view. Due to this flattening, the egg usually comes to rest upon a plane surface so that the pole is either above or below. However, when centrifuged in a supporting medium such as sucrose, the egg orients in the centrifuge tube with the polar axis perpendicular to the axis of centrifuging. If a centrifugal force of about 66,000 times gravity is applied for 60 to 80 minutes, the interior protoplasm becomes stratified and the egg elongates into a dumb-bell form, but the unactivated egg does not separate into two clean-cut fragments with the forces that have been used thus far. It is a striking fact that the neck of the dumb-bell is not circular in cross-section. When

the egg is rotated upon its long axis one observes that the neck of the dumb-bell is two or three times wider than it is thick. This is undoubtedly due to the shape of the non-centrifuged egg and to its orientation in the centrifuge. Consequently, if the polar axis has not been displaced, it should extend transversely across the neck of the dumb-bell.

The zones of stratification of the centrifuged egg are as follows: at the centripetal pole are the large oil drops; next a layer of hyaline protoplasm; an indistinct layer of fine granules; a second hyaline zone, occasionally lacking; and a zone of small, heavy granules, at the center of which is a vortex of granules of slightly different properties. The large germinal vesicle with its contained nucleolus lies in the hyaline and finely granular zones.

To obtain egg fragments, these dumb-bells, after removal from the centrifuge, were cut by hand with microneedles. The vitelline membrane was not cut by this process, but the protoplasm was pinched into two fragments. It was easiest to cut across the neck of the dumb-bell, excluding most or all of the yolk from the light fragment. Such a cut will be termed the "upper" cut. Cuts were also made at the lower edge of the yolk zone and will be referred to as the "lower" cuts. "Intermediate" cuts are those passing between "upper" and "lower", across the yolk zone.

If the eggs are not cut after removal from the centrifuge, the dumb-bell changes fairly rapidly to an ellipsoid, and the egg, if uninseminated, remains in this condition with persistent stratification for more than 24 hours. If the cut eggs are not inseminated, neither fragment is activated, and neither fragment develops.

Upon insemination, either fragment, or both, may be activated. Activation is indicated by the separation of the fragment from the vitelline membrane, by the formation of the perivitelline space due to the extrusion of the cortical jelly, and by the partial rounding up of the fragment. Activated light fragments usually cleave, and after some hours reach a multicellular state. The heavy fragments, even though activated by the sperm, never cleave. Out of approximately 500 cut eggs not a single case of cleavage in a heavy fragment has been observed. This is independent of the position of the cut. In the elongate whole centrifuged egg, however, the centrifugal region cleaves, and since the stratification persists to some extent during development we may conclude that it is not the high concentration of granules alone that prevents the cleavage of the heavy fragment.

The light fragment invariably contains the germinal vesicle, while the heavy fragment does not. The classical experiments of Delage and of E. B. Wilson have demonstrated the importance of the germinal vesicle for fertilization and development in the eggs of other marine forms. The eggs of Asterias and Cerebratulus are non-fertilizable in the germinal vesicle stage, and it was shown by these workers that fragments taken from an egg with intact germinal vesicle are non-fertilizable. But non-nucleated fragments taken from an egg in which the germinal vesicle has begun to break down, may be fertilized and develop. The Nereis egg differs from the above mentioned forms in that the whole egg is normally fertilizable in the germinal vesicle stage.

In regard to the development of the light or centripetal fragment of the Nereis egg, a relationship exists between the position of the cut, and the development or non-development of the ciliated larva. In the developing centrifuged whole egg, the prototrochal cilia make their appearance 10 or 12 hours after insemination. In the large centripetal fragments produced by the "lower" cut, development usually proceeds rapidly to a multicellular condition with cells of varying sizes, and certain of these cells develop cilia as in the whole centrifuged egg.

If we follow the development of the small centripetal fragments that have been produced by the "upper" cut across the neck of the dumb-bell, we find that cleavage proceeds more slowly and less regularly, and while the multicellular condition may be attained these embryos almost never develop cilia. These conclusions are based upon isolation cultures as well as upon mass cultures of centrifuged cut eggs. Of 103 isolated cut eggs 83 developed far enough to be included. Of 42 isolated "upper" light fragments, 3 became ciliated; of 20 "intermediate" light fragments, 9 became ciliated; and of 21 "lower" fragments, 14 became ciliated. A few of the "lower" light fragments developed the brown pigment associated with the prototrochal band, eye-spots, anal pigment, and in one case, setae. Apical tufts have not been found in these strongly centrifuged eggs.

These experiments indicate that some material substance in the vicinity of the equator of the strongly centrifuged Nereis egg is essential for the development of a larva bearing prototrochal cilia. Whether this material has been displaced into this position by the centrifugal force, or whether it has retained its relationship to the polar axis despite the centrifuging, is a problem which remains to be solved.

In addition, evidence has been presented which indicates that material in the centripetal region of this egg is essential for fertilization. It is very probable that this material is associated with the germinal vesicle.

(This article is based upon a seminar report given at the Marine Biological Laboratory on August 16.)

A MICRODISSECTION STUDY OF AMPHIBIAN CHROMOSOMES

DR. W. R. DURYEE
Washington Square College, New York University

Synaptic chromosomes in the germinal vesicles of ovarian eggs of the salamander, *Triturus pyrrhogaster,* appear to be the largest yet observed. At one stage two pairs are over 500μ long in the relaxed condition. During the years of growth synaptic pairs are shown by microdissection to be fused with one another at the chiasmata.

Toward the end of ovarian development chromosomes become much shorter, consisting of transparent gelatinous cylinders in which denser less elastic segments are imbedded. Consequently in this condition vertebrate chromosomes are comparable in structure to those of the dipteran salivary gland.

However amphibian chromosomes go through a protracted growth period (in anurans lasting over a year) during which each nodal segment gives off elastic granular side branches (averaging 30μ) in the form of loops. Since these loops are sloughed off from the chromonema cylinder, not being resorbed under either normal or experimental conditions, we have a concrete example of material being synthesized under the direct influence of each chromomere. Such new material is added to the cytoplasm at the time of germinal vesicle breakdown, if not before.

It has been found that the relative tensile strength of the chromonemata drops from an average increase of 354 per cent in Ca-free Ringer to about 102 per cent when $CaCl_2$ 0.001 molar is present. Under the latter condition chromosomes become more brittle, and tend to come apart at the chiasmata.

(This article is based upon a seminar report given at the Marine Biological Laboratory on August 16.)

DEPARTMENT OF PUBLICATIONS

THE METABOLISM OF LIVING TISSUES. By Eric Holmes. Cambridge: At the University Press. New York: The Macmillan Company.

The author points out in his preface that the book is primarily intended for use by students entering the subject of biochemistry. It is not meant to replace the textbooks on this topic but rather to supplement their detailed and systematic descriptions of compounds and reactions of biochemical interest by a method of treatment which "renders it easier to visualise the active, dynamic aspect of tissue metabolism."

There can be little doubt that the book will adequately serve the purpose for which it was written. Hardly a more pleasing compliment could be paid to the author of the small volume than to say that not only the beginner but everyone theoretically or experimentally interested in biochemistry is bound to find here instruction and stimulation. The fluid style and the conversational form of the presentation prevent fatigue and keep the interest of the reader alive.

The subject matter dealt with is best summarised by giving the headings of the chapters of the monograph: Enzymes, Some General Features of Cell Metabolism and Methods of Investigation, Oxidations, Oxidation-Reduction Potential, Nitrogen Metabolism and Carbohydrate and Fat Metabolism of the Liver, The Kidney, Voluntary and Cardiac Muscle, The Nervous System, The Correlation of the Metabolism of different Tissues, The Hormones, The Influence of some Inorganic Substances, The Vitamins.

The reader will soon become aware of the fact that the author's main personal interest centers on problems of the metabolism of isolated tissues of the kind commonly studied by means of the technique of tissue slices and the manometric methods developed by Otto Warburg and his colleagues.

It is here that the author writes with full authority. One will recall that he was able to demonstrate, in a recent paper appearing in the *Biochemical Journal,* for the first time an effect of insulin on isolated tissues. The effect consists in an inhibition of the deamination of alanine and of its subsequent conversion into carbohydrate in liver slices. But not for this reason alone will his presentation of carbohydrate metabolism in isolated tissues and of the role of insulin and other hormones in relation to carbohydrate metabolism be appreciated. This subject and even more complex problems like the nature of the so-called Pasteur-Meyerhof effect are treated with a rare lucidity and with a truly critical attitude.

Questions related to chemical structure and physical chemical considerations appear to be somewhat outside the focus of the author. In order to be complete in his presentation he has obviously filled gaps in his personal experience by rehashing text book material. To go into details here would mean to detract from the indisputable merits of the book.

Ordinarily it is considered to be the unpleasant duty of the reviewer to bring minor slips and misprints to the attention of the author. Most of these seem to have crept into the manuscript in the process of typing and undoubtedly the author will, in the meantime, have become aware of them. The reviewer has therefore resolved to skip this part of his task.

—*Kurt G. Stern.*

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris Gorokhoff and Hazel Goodale.

Entered as second-class matter, July 11, 1935, at the U. S. Post Office at Woods Hole, Massachusetts, under the Act of March 3, 1879, and re-entered July 23, 1938.

GENETICISTS AT WOODS HOLE

Biologists at Woods Hole look forward at this time of year to the influx of geneticists who come to attend the summer meetings of the Genetics Society of America. The gatherings during the first three days of September mark the fifth anniversary of the annual meetings at the Marine Biological Laboratory.

Woods Hole has for years been a center of investigations in genetics as in other branches of biology. This may have seemed to some a bit out of place, for marine forms are not in general favorable material for genetic investigation and consequently they have not been utilized in this connection. The attractions of Woods Hole for geneticists are in part the same as for other people—the climate, the swimming and the beach parties, which, added to the facilities of the laboratory, make the place an ideal location both for work and for play.

But geneticists are finding more and more of real value for their science in association with biologists working in other fields. In earlier years it was essential that genetics should develop according to its own specialized methods until a body of principles of heredity *sensu stricto* was constructed. At that time ratios of traits in fraternities and pedigrees were of more interest than the traits themselves. More recently geneticists have moved out into adjoining fields. It is now a question whether developmental and physiological genetics are not merely the application of knowledge of genetics to certain problems of embryology and physiology.

The meetings here offer an opportunity of increasing mutual advantage both to the visitors and to the resident biologist. The embryologists and the physiologists may find some of their problems more readily solved by a genetic approach, while geneticists will need to know more and more of embryology and of physiology in order to comprehend the nature of the gene and of its action in the causation of hereditary differences.

The increasing success of the genetics meetings at Woods Hole is due in large measure to the energy and enthusiasm of its present local representative, Dr. P. W. Whiting, who was secretary-treasurer of the Society when it first convened in Woods Hole (1934), and who was its president in 1936.

Introducing

Manfred Kiese, Assistant in Pharmacology, University of Berlin; Rockefeller Foundation Fellow at Harvard Medical School and at Woods Hole.

This is Dr. Kiese's first trip to America and his first opportunity for doing research outside of Germany. He was born in Stettin, studied at the Universities of Hamburg, Frankfurt and Munich and finished the work for his M.D. degree at Berlin. He has been at Berlin since then, for a short while at the Medical Clinic, and during the last three years at the Pharmacological Institute where he is an assistant in pharmacology. The first piece of research that he did was on chronic arsine poisoning and the effects of noxious gases. He then undertook investigations of the pharmacological effects of adrenaline-like substances on the bronchial muscles, and especially on the circulation. He also investigated the mode of distribution of cardiac glucosides between the heart and other organs.

In addition to papers reporting results of these investigations, further publications include pharmacological studies of coffee and caffeine, the influence of caffeine on the metabolism of the mammalian heart and the study of the action of organ extracts on the circulatory system and especially on the heart.

Dr. Kiese's most important study has been on the relation between mechanical work, size and metabolism of the mammalian heart. In this study it was shown that the energy set free by the heart muscle during contraction depends not only upon the fiber length but also on the amount of work done (Fenn-effect). Further studies included work on the effect of ephedrin and related substances on mammalian heart action and metabolism and studies on methemoglobin and the hemoglobin-methemoglobin system.

Interest in the mechanism of pharmacological action led Dr. Kiese to study more intensively the biochemical aspects of these problems. He worked in the biochemistry laboratory at Harvard with Dr. A. B. Hastings. There he investigated the oxidation of SH-compounds and the influence of oxidation and reduction on the activity of hydrolytic enzymes At Woods Hole he has been working on carbonic anhydrase. Since it is necessary to keep these experiments at constant temperature he often spends a hot afternoon in the "cooler"!

He seems not to be bothered by the cold, for his favorite sport is a winter one; skiing in New Hampshire last year occupied many of his week ends, and he hopes to stay long enough to ski again before returning to Germany. Dr. Kiese will leave America when the work in hand is finished, probably around Christmas time.—*M. F. M.*

ITEMS OF INTEREST

DR. JACINTO STEINHARDT, Rockefeller Fellow and for five years instructor in general physiology at Columbia University, has been appointed physical chemist at the Bureau of Standards with a group supported by the Textile Foundation working on the properties and structure of protein fibers.

DR. CHARLES D. HOWELL was recently appointed instructor in biology at Middlebury College, Middlebury, Vt., to succeed the late Professor Raymond L. Barney who died on July 12th. Dr. Howell was formerly professor of biology at Elizabethtown College, Elizabethtown, Pa.

DR. C. P. KRAATZ, instructor in physiology and pharmacology at the Chicago Medical School spent Tuesday and Wednesday of this week at Woods Hole.

DR. HENRY J. FRY, investigator in cytology at the Cornell Medical School, and MR. ROBERT DUFFUS, of the editorial staff of the *New York Times*, left at the end of this week to take a canoe trip on Martha's Vineyard. They will explore the Vineyard in the neighborhood of Cape Poge and plan to be camping out along the shores of the Vineyard for six nights.

DR. BASILE LUYET, professor of biophysics at the St. Louis University School of Medicine, is now on a trip to Europe. He delivered a paper entitled "Water and the ultra-structure of protoplasm" at the Fifth International Congress of Experimental Cytology at Zürich. He has also delivered two other papers.

SUPPLEMENTARY DIRECTORY

Beck, L. V. res. fel. biol. Pennsylvania Med. Lib.
Brambel, C. E. instr. zool. Hopkins. Br 301.
Diller, Irene C. res. assoc. zool. Pennsylvania. Br 219. A 301.
Diller, W. F. asst. prof. zool. Dartmouth. L 34. A 301.
Pipkin, C. A. Texas. Lib.
Scholl, S. M. res. asst. zool. Toledo. Br 315.

DATE OF DEPARTURE OF INVESTIGATORS

Birnbaum, S. M.	August 22
Cooper, K. W.	August 18
Cooper, Ruth S.	August 18
Dugal, L. P.	August 22
Drouet, F.	August 16
Goodrich, H. B.	August 16
Harrold, C. M.	August 16
Hatch, Cleora	August 17
Hill, E. S.	August 20
Karady, S.	August 21
Kreezer, G.	August 22
Lincheid, Martha	August 22
Miller, James	August 13
Morrill, C. V.	August 16
Mullins, L. J.	August 5
Musser, Ruth E.	August 17
Weiss, P.	August 16

DR. AND MRS. D. M. WHITAKER left last Friday morning by automobile for their home in Palo Alto, California. They are leaving their two children with their grandparents Dr. and Mrs. T. H. Morgan with whom they will return to California on September 9th.

Exhibit of Marine Flora and Fauna of Woods Hole

The annual exhibitions by the Botany department and Invertebrate class took place in the main lobby of the M. B. L. Dr. W. R. Taylor was in charge of the botany exhibit, which was made by Dr. Hannah Crosdale, Bettina Bien, and Gladys Bulmer and included some ninety-seven specimens all of which were collected within the distance of a half-hour's trip from Woods Hole, i.e. Nonamessett, The Spindle, Penzance salt marshes and the Eel Pond. The exhibit contained no unusual specimens but was remarkable in that it was very complete, although made within such a short distance of Woods Hole.

The Invertebrate class exhibit came from Hadley Harbor, known for its particular style of mud baths and was in the care of volunteers under the direction of John Wightman of Brown University, instructor in the Invertebrate course. In it were some unusual specimens. One was *Lepas pectinata*, a goose barnacle, not indigenous to this locality but generally found in the Sargasso Sea from which it may have been carried by the Gulf Stream. It was found attached to sea weed. Two other specimens which are uncommon were the mollusc, *Barnea truncata*, a wood boring form, and the arthropod, *Callianassa atlantica*. About 150 specimens were taken but many of them died soon after being put on exhibit, while others were too small for exhibition.

CURRENTS IN THE HOLE

At the following hours (Daylight Saving Time) the current in the Hole turns to run from Buzzards Bay to Vineyard Sound:

Date	A. M.	P. M.
August 27	6:06	6:31
August 28	6:54	7:15
August 29	7:40	8:08
August 30	8:24	8:58
August 31	9:17	9:51
September 1	10:08	10:46
September 2	11:02	11:41
September 3	11:57	
September 4	12:43	12:59

In each case the current changes approximately six hours later and runs from the Sound to the Bay.

PARTIAL LIST OF PAPERS AND DEMONSTRATIONS PRESENTED AT THE GENERAL SCIENTIFIC MEETINGS TO BE HELD ON AUGUST 30 AND 31

Papers Presented in Person

Algire, Glenn H. Cytological studies on the living thyroid of the salamander.

Bowen, William J. The effect of vanadium on the rate of growth in *Chilomonas paramecium.*

Bedichek, Sarah. Sex balance in the progeny of triploid Drosophila.

Cheney, Ralph H. Micro-structural changes in muscle fibers after caffeine.

Cole, Kenneth S. and Curtis, Howard J. Electric impedance of nerve during activity.

Dugal, Louis-Paul and Irving, Laurence. The relation of the shell to anaerobic metabolism in *Venus mercenaria.*

Dziemian, A. J. and Parpart, A. K. Permeability and the lipoid content of the erythrocyte.

Elftman, Herbert. The function of muscles in locomotion.

Geldard, Frank A. The vibratory response of the skin and its relation to pressure sensitivity.

Harvey, Ethel Brown. Development of half-eggs of Chaetopterus obtained by centrifugal force.

Henshaw, Paul S. (1) The radiosensitivity of Arbacia eggs following fertilization. (2) The question of whether the delay in cleavage of Arbacia eggs produced with X-rays is caused by a general slowing of the cleavage process or a block at some particular stage.

Maloeuf, N. S. R. (1) On the kidney of the crayfish and on the uptake of chlorid from fresh water by this animal. (2) The osmoregulative function of the alimentary tract of the earthworm and on the uptake of chlorid from fresh water by this animal.

Melland, Amicia M. Isolation of salivary gland nuclei.

Pond, S. E., Little, E. P., Smith, A. M., and Graham, J. D. A comparative study of water aspirators.

Richards, O. W. and Hawley, Katherine J. Mold elimination in marine laboratories.

Speidel, Carl C. Some features of contraction nodes and retraction clots as observed in single fibers of cardiac and skeletal muscle of both vertebrates and invertebrates.

Sandow, Alexander and Kenneth Moritz. Tension output of muscles in hypotonic solutions.

Turner, John P. Mitochondria and other inclusions in the ciliate Tillina canalifera.

Uhlenhuth, Eduard. The antihormone problem in the salamander.

White, Michael J. D. The heteropycnosis of sex-chromosomes and its interpretation in terms of spiral structure.

Wolf, E. A. Reversal of phototropic reaction in Daphnia by use of photosensitizing dyes.

Papers Read by Title

Armstrong, C. W. J. and Fisher, K. C. The effect of sodium azide on the frequency of the embryonic Fundulus heart.

Cooper, Ruth Snyder. Probable absence of a chromatophore activator in *Limulus polyphemus.*

Elwyn, Adolph. The melanophore expanding activator of the ascidian neural gland.

Karady, Stephen. Alarm reaction and adaptation syndrome on lower vertebrates (*Fundulus majalis*).

Stannard, J. N. The effect of sodium azide in the respiration of frog muscle.

Waterman, A. J. Respiratory stimulants and gastrulation in Arbacia.

Wolf, Opal M. (1) Oviducts of pituitary stimulated female *Rana pipiens.* (2) Mitotic activity of the islands of Langerhans and parathyroids of rats following pituitary and colchicine injections.

Demonstrations

Algire, Glenn H. The apparatus for the cytological study of the thyroid in the living salamander.

Cole, Kenneth S. and Curtis, Howard J. Electric impedance changes in the squid giant axon following excitation.

Galtsoff, Paul S. (1) Sex reversal in adult oysters. (2) Method of measuring and recording rate of flow of water through the gills of the oyster. (3) Respiration of the oyster.

Henshaw, Paul S. Cellular abnormalities produced by X-rays.

Pond, S. E., Little, E. P., Smith, A. M. and Graham, J. D. Demonstration of pumps both foreign and domestic (commercial and some made at M. B. L.) and method in use for study.

Sichel, F. J. and Pond, S. E. Multicontact Lucas rheotome. Room 6 Old Main.

Pond, S. E. and Little, E. P. Water aspirator tests and comparisons. Room 308 Br.

Speidel, Carl C. Motion picture showing microscopic changes in fibers of cardiac and skeletal muscle of invertebrates and vertebrates during contraction, retraction and clotting. (2 reels, 30 minutes.)

White, Michael J. D. Spiral structure of animal chromosomes (photographs).

INVERTEBRATE CLASS NOTES

"Hmm, it doesn't seem to me that the *Winifred* can navigate in such shallow water." This was a bit of invertebrate kibiting as the *Winifred* was ready to discharge its passengers at the start of Saturday's Lagoon Pond Bridge field trip. And the kibiters were right, for the *Winifred* was aground. Puffing and snorting, she backed up only to repeat the performance. The *Nereis* had to come to the rescue this time and, amid the snorts and chuckles of the scoffing invertebrates, the *Winifred* again returned to deeper waters. A few other tragedies were narrowly avoided—some members of team three escaped the decapitation threatened by a low crossbeam of the bridge as they were passing under it in a rowboat; team five's bucket tossed on Neptune's lap for a while until a member of team one reached out and picked it up; and there were six tired angels on the verge of a nervous breakdown due to the rapid fire checking necessary to keep up with the number of specimens found.

Sunday evening after a long and arduous day trying to find some almost microscopic ganglia in a beast named Busycon, a feast of boiled corn properly buttered and salted and a minute sector of homemade apple pie was most welcome Everyone sat around contentedly munching corn and smearing fully fifty per cent of it over their faces while the Busycon enjoyed a respite from the incessant and ineffectual poking to which he had been subjected during the day. The corn, it might be added, was what remained of a generous supply of food taken on The Collecting Net's outing to Penikese and Cuttyhunk which was supported by quite a few inverts.

The South side of the laboratory is beginning to redeem itself on the baseball field. Although they lost the game on Thursday to the tune of eleven to seven, that score was a lot better than the previous crushing defeat. And they managed to tie the score of Tuesday's game at twelve. Vague rumors are heard concerning their winning the next game. A new element was introduced into the baseball situation on Fridav when the girls of the class lost to the boys, who were playing left-handed, by the score of six to seven.

There had been a general feeling of futility creeping over the invertebrate class due to the fact that, although they could recognize minute animals growing along the shore and under the surface of the water, most of the class didn't know a rowboat from a schooner. Dr. Crowell's Chalk Talk on the Sailing Rigs Common to the Woods Hole region the evening of August 17 remedied this dreadful state of affairs pleasantly and most competently. —*Elizabeth L. Jordan*

The New Tide Gage House

The tide gage house of Woods Hole Oceanographic Institution is now being reconstructed. Originally built by the Oceanographic Institution on its wharf to house the standard automatic tide gage, it has been operated in co-operation with the Coast and Geodetic Survey. Recently a PWA allotment was made to the U. S. Coast and Geodetic Survey for the reconstruction of its primary tide gage houses along the Atlantic, Gulf and Pacific Coasts, which work is to be started during the latter part of August. The Woods Hole tide gage is now being rebuilt by Mr. Forest E. Boynton, of Woods Hole, and will be insulated against moisture in such a manner as to make its interior much dryer than it has been in the past. This will result in better operation of the tide gage and will give more satisfactory tidal records.

HYDROGRAPHIC SURVEY OF WOODS HOLE HARBOR AND NANTUCKET SOUND

Lieut. Charles M. Thomas
Commanding U. S. C. & G. S. Motor Vessel Gilbert

A resurvey of Woods Hole Harbor and its approaches, and also of the northern half of Nantucket Sound, is being made this season by the U. S. Coast and Geodetic Survey Motor Vessel *Gilbert,* which arrived at Woods Hole last May.

The last survey made of this area was almost fifty years ago, from 1888 to 1890, by parties from off the Coast Survey Schooner *Eagre*. This was long before the time when the modern method of making similar surveys was developed. At that time all hydrographic surveys were made with either the lead-line, or the sounding machine where the depths were too great to obtain them with the lead-line. A boat proceeding at the proper sounding speed of about six knots, could, with the desired degree of accuracy, obtain only about four soundings per minute in depths of two fathoms or less, and only one sounding per minute where the depths are between ten and fifteen fathoms (60 to 90 feet). However, with the Dorsey shoal water fathometer, now installed on most of the vessels of the Coast and Geodetic Survey, continuous soundings are obtained by the echo method at the rate of twenty per second, the depths being indicated by a flashing neon light on the dial of the fathometer. However, these sound-

ings are recorded only about every ten or twenty seconds, depending upon the depths, although irregular soundings showing unusual shoals or depths are recorded at odd intervals of time. A sounding vessel with a speed of ten knots obtains continuous fathometer soundings about every four inches along the bottom, whereas with a fast leadsman, lead-line soundings could be obtained only about every seventy-five yards along the sounding line.

With the fathometer, soundings can actually be obtained more accurately than they can with the lead-line, especially where there are strong currents. Since sound travels at the rate of about 4,920 feet per second in sea water, (the exact velocity depending upon the temperature, salinity and pressure of the water), or about four and a half times as fast as sound travels in the air, it seems remarkable that the time of the bottom echoes as measured by the fathometer, and automatically converted into depths, has been determined to such a refined degree of accuracy. In calm waters correct fathometer depths can be determined within a small fraction of a foot, and these instruments are now being used as temporary tide gages, by some of the vessels of the Coast and Geodetic Survey, to determine the relative time and range of the tide in off-shore areas. On such occasions the vessel has to be anchored over a flat bottom for about twelve hours or more, and the changing depths recorded about every ten or fifteen minutes.

The southern limit of this project is to include Hedge Fence and Horse Shoe Shoals, and Handkerchief Lightship, the survey extending as far eastward as Monomoy Island.

Fathometer soundings are taken over the areas sufficiently deep for safe navigation of the Motor Vessel *Gilbert*. A hydrographic launch, and also a dory are used for obtaining the depths over the numerous shoals and for the inshore areas, the lead-line being used for determining the soundings.

While the hydrographic survey is being made it is necessary to know the correct position of the vessel at least every two or three minutes while running the parallel sounding lines, which vary from fifty to two hundred meters apart, depending upon the depths. Prominent objects, such as water tanks, church steeples, and various survey signals of different descriptions for easy identification are located along the shore by theodolite angles, their latitudes and longitudes computed, and these stations plotted on the survey sheet, known as the "boat sheet". The positions of the survey vessel are then determined by obtaining two sextant angles simultaneously taken from the boat to three shore signals easily seen and identified. These angles are set on a three-arm protractor, after having been recorded, and this instrument is shifted around on the boat sheet until the three arms coincide with the three signals used, the center of the protractor locating the boat's position at the instant the angles were taken. This procedure is continued throughout the season.

At the end of the season neat and accurate smooth sheets are plotted up of all the survey work accomplished, and forwarded to the Washington office, where they are checked, reviewed and inspected. If found satisfactory they are approved and turned over to the Cartographic Section of the Chart Division, from which the characteristic soundings are selected for the final nautical chart, which generally includes a very small percentage of the thousands of soundings actually taken and recorded. After going through various processes of preparation, it is finally turned over to the Printing Section, and emerges from their presses as the finished product, another one of the numerous nautical charts of the U. S. Coast and Geodetic Survey, which charts have for almost one hundred years proved of untold value towards the protection of life and property of the many wise mariners who use them properly while navigating the coasts and harbors of the United States and its insular possessions.

THE INFLUENCE OF FLUCTUATIONS IN THE MAJOR OCEAN CURRENT ON THE CLIMATE AND THE FISHERIES

(Continued from page 145)

as these that the Royal Society had in mind when two years ago a special committee undertook to raise some money to develop the Bermuda Biological Station as an oceanographic base. The cooperation of the Woods Hole Oceanographic Institution was enlisted and a five year program of investigation has already been in progress for over a year. One satisfactory result of all this is that the Bermuda Laboratory has indeed received much needed financial support. The fact that a small research vessel has been provided by the Royal Society makes us hope that Bermuda will become more and more used by biological investigators. It is much less certain that the physical program, part of which will be outlined here, will come anywhere near the desired goal.

In studying the fluctuations of a major ocean current such as the Gulf Stream, it is of course necessary to secure observations over a considerable period of years before it can be said with

certainty whether or not the current does have a significant annual variation in strength. Our temptation is to assume that it does. It may very well be that the Gulf Stream runs as steadily as a clock, in which case the various theories presented below have little practical application. However, it sometimes pays to speculate, and physical oceanography has now reached the stage in its development when speculation can be put on a reasonably sound physical basis.

The popular belief, of course, is that the Gulf Stream fluctuates quite widely in strength and position. The available modern observations indicate that this is certainly not correct. However, it can be said that the Gulf Stream probably has a tendency to vary in strength from time to time by about 10%. Perhaps even such slight fluctuations in the current can have quite far reaching consequences.

It is always easier to discuss a particular problem than to generalize. Therefore, the Gulf Stream will be used to illustrate the principles to be explained below. However, the Gulf Stream is only one part of the main North Atlantic anticyclonic eddy and each of the other oceans has a corresponding eddy. In other words, if our analysis of the problem in the case of the Gulf Stream is sound, the same principles can be applied to other parts of the world.

There are three main theories that have been advanced by scientists to explain the influence of the Gulf Stream on the climate and fishery of Northern Europe. In the light of recent oceanographic observations none of these theories is entirely satisfactory. Nevertheless, it will be worth while to state these theories for they constitute the background for the fourth and newest theory that is now being developed.

The oldest theory and the one most usually subscribed to simply assumes that the Gulf Stream resembles a river of warm water flowing northeastward towards the European coast and that it varies in strength from year to year. When the current is strong, sea surface temperatures will tend to rise off the Irish coast, for example, and if this trend persists, the climate will become milder. If the warm saline water floods in over the continental shelf or enters the North Sea in exceptional quantities, the fishery will be affected.

The chief difficulty with this theory is that as a transporter of warm water the Gulf Stream is in reality relatively ineffectual and that the greater part of the current does not flow towards the northeast, but curves around to the right towards the Azores and is there picked up by the northeast trades to continue in the anticyclonic eddy of the North Atlantic basin. A northern branch of the Gulf Stream, it is true, does carry some relatively warm water eventually to the Norwegian Sea. The fluctuations appear to be much greater in this northern branch than they are in the main eddy and, as will be argued presently, it may well be that even the relationship is reversed and that the northern branch strengthens when the Gulf Stream and the North Atlantic eddy weaken. In short, the early theory emphasizes the fact that from the European standpoint the Gulf Stream appears to be a variable current, but the theory is in too simple a form to be helpful without considerable modification.

The second theory which attempts to explain the variations in the temperature and salinity of the European coastal waters was advanced by a French oceanographer, LeDanois. This theory minimizes the importance of the Gulf Stream, but stresses the fact that from the European standpoint widespread invasions of warm water apparently occur from time to time. It is known as LeDanois' transgression theory.

LeDanois draws attention to the fact that on the whole the waters of the North Atlantic can be subdivided into two relatively independent parts on the basis of temperature and salinity. At the surface these two contrasting water-masses meet in a line extending roughly from the southern Grand Banks to the Faroe Islands. South of this boundary is found at the surface a wedge shaped layer of relatively warm water having salinities higher than 35%, the thickness of the wedge increasing towards the south. To the north and at all depths below the light saline water lies a much greater mass of colder water with salinities always lower than 35%. LeDanois believes that the wedge-shaped saline mass is not constant in position or volume. An expansion of the wedge towards the northeast he terms a transgression and a retreat of the warm water he calls a regression. He has found evidence that the transgressions occur in regular cycles having periods of 1, 4½, 9 and 18 years. The regularity of the cycles he attributes to astronomical causes.

Many objections can be raised against the details of the transgression theory and especially the modern oceanographic observations do not support LeDanois' assumption that the Gulf Stream does not continue east of the Grand Banks. Nevertheless, there is reason to believe that the warm saline water which from time to time seems to invade the European costal areas arrives more as a tongue than as a current. However, as yet there is little sound basis to the idea that the transgressions occur in regular cycles.

The third theory and perhaps the one in which fewest flaws can be found, is based on the assumption that the winds are the primary variable. During years when on the whole southerly winds prevail across the belt between the Grand Banks and Ireland, the surface layer will be shifted to

the north and, therefore, at any one latitude sea surface temperatures will rise. In the same way, if winds with northerly components predominate, colder water will be brought into the critical area off the European coast. A general change in sea surface temperatures of only a few degrees produced in this manner should result in a significant climatic variation. Likewise an invasion of oceanic water over the fishing grounds might be caused by a few exceptional westerly gales. Unfortunately no suitable study has yet been made of the variability of the winds over the critical area between the Grand Banks and the Irish coast. All that we can say now is that if the winds are sufficiently variable, they probably are capable of producing fluctuations through shifts of the surface layer without necessarily upsetting the permanent deeper circulation. During a considerable part of the year the surface layer is well separated from the rest of the ocean by a sharp thermocline which may allow it to move quite independently.

We will return to the frictional effect of the wind, but first the newest theory, in which the strength of the whole North Atlantic eddy is considered the primary variable, will be discussed.

For this theory to be understood, it is necessary to have some knowledge of the general principles of Bjerknes' circulation theorem. In the first place, it must be realized that for any moving body to continue in a straight path in the geographical sense, it must be acted upon by a force that in the northern hemisphere pulls it to the left. In other words, because of the earth's rotation a given mass of water will follow the course of an inertia circle unless it experiences some other force. In ocean currents it is the pressure gradient that exactly counteracts the apparent deflection due to the earth's rotation. The pressure gradient and the fictitious deflective force are both at right angles to the direction of flow. Therefore, the current flows across the pressure gradient and not down it as would be the case if the earth were not rotating.

Since the diameter of an inertia circle varies with the velocity of the moving particle (and also with the latitude), in general the greater the velocity of a current, the greater must be the pressure gradient at right angles to its course. In the case of the Gulf Stream, for example, this means that as the current increases, the thickness of the warm water in the Sargasso Sea must also increase, and that as the current decreases, the warm layer on its right hand side must become shallower. In other words, in the main anticyclonic eddy of any ocean, the thickness of the warm central core will vary with the velocity of the surrounding circulation.

Now the main superficial, oceanic currents derive their energy from the anticyclonic wind systems of mid latitudes. If the strength of the winds vary, the circulation theorem demands that either the volume of warm water at the center will have to change or the eddy must change in diameter. With decreasing currents and, therefore, decreasing pressure gradient, either the eddy will have to expand or warm water will have to be discharged. The third possibility, i.e., cooling at the center accompanying a decrease in the wind system, probably does not occur to any marked extent. In the same way, a strengthening of the eddy will in theory result in a decrease in its diameter. This in brief is the underlying principle of the fourth and newest theory concerning the influence of fluctuations in the major ocean currents.

As mentioned already, the theory assumes that the strength of the North Atlantic eddy varies slightly from year to year. If this is so, then it can be argued that since the eddy is not free to expand because of the limitations of the basin, a decrease in strength of the Gulf Stream will be accompanied by a discharge of warm water towards the northeast. The amount of warm water which must be discharged as the result of a 10% decrease in transport by the currents is surprisingly large. A simple calculation will show that in this event the northern tongue of warm water that moves towards the European coast will be strengthened by about 225,000 cubic kilometers. In the same way, as the currents increase, the northern branch will be interrupted until this amount of warm water has accumulated in the Sargasso Sea. We have here a mechanism, it is believed, that can explain how relatively steady currents can cause quite wide variations in the temperature and salinity of the waters off the coast of northern Europe. As mentioned already, the relationship is quite the reverse from that popularly believed in. As the Gulf Stream decreases in strength the climate of Great Britian, for example, should become milder and her shores should be bathed by relatively warm saline water. When the Gulf Stream increases the reverse should happen.

At the present time the Woods Hole Oceanographic Institution is cooperating with the Bermuda Biological Station to collect routine observations that will prove which of the theories we have mentioned is the correct one. If this can be accomplished, it is not beyond the bounds of possibility to suggest that by means of a few simple observations the major trends in the British climate and fishery could be forecasted.

Because of the prevailing westerly winds at the present time it seems very improbable that the fluctuations in strength of the Gulf Stream can affect the climate of the eastern coast of the United States. However, they may well influence our fishery, especially northeast of Cape Hatteras.

Of course, the fluctuations of the offshore currents are not the only cause of the success or failure of a given brood of young fish. The local hydrological situation can also play an important part. However, as will be explained below there are at least two mechanisms whereby the Gulf Stream can seriously disturb the waters over the continental shelf.

The first mechanism again involves the circulation theorem. It is chiefly important to bottom fisheries near the edge of the continental shelf. As the current increases in strength, the depth of the main thermocline on its left hand side must become less. When the current decreases, all the isotherms of this layer of relatively sharp vertical temperature gradient will lie deeper. Thus the bottom temperatures on the continental slope will vary with the strength of the Gulf Stream. At depths of about 200 meters a small change in the current can in this way result in a marked change of bottom temperature.

The second mechanism is less clearly understood and presumably it is most vital to the fish population just after the spawning period. It is a mechanism whereby a whole year class of fish can be wiped out completely in a few days.

One of the hydrographical problems which is very difficult to understand involves the tendency for coastal water to remain over the continental shelf. Our coastal waters are less dense than the offshore water. The prevailing wind is from the west. Why are our coastal waters not continually drifting out to sea? To take a specific case, why does Georges Bank water remain over the bank? If it drifted away, the whole population of young fish in the pelagic stage would be lost. As a matter of fact Walford, who has studied the plankton tows from 1932, finds that in that year practically all the young haddock were carried off the bank and consequently the 1932 year class is very weak in the present haddock population. Fortunately for the fishery such catastrophes do not happen frequently. If they did, no young fish could survive because all of the important commercial fish go through a planktonic stage when they cannot swim against the currents. This critical stage lasts from a few weeks to several months depending on the species.

The zone between the Gulf Stream and the 100 fathom curve is a mixing zone for coastal water and warm saline water that has escaped north of the Gulf Stream. The mixing agent is the eddies that are formed along the northern edge of the current. This zone with mixed water at the surface is called the slope water band. It extends from Cape Hatteras to the Grand Banks and averages 60 to 100 miles in width. The eddies in the slope water band seem to be of two main types. There is the type which persists for several weeks or months and which does not assimilate much coastal water. There is also the other type which is frictionally driven and in which can be observed a definite tongue of coastal water which is being sucked into the whirl. This type, apparently, is very dangerous to the fish population during its planktonic stage.

Since such an eddy also contains a tongue of warm saline water from the Gulf Stream, we are in hopes that the formation of these eddies is caused by fluctuations in the strength of the current and the necessity of a discharge of the warm water from the Sargasso Sea as mentioned previously. If this is the case such destructive eddies should be rare when the Gulf Stream is increasing in strength and should be common during years when the current is weakening. To this extent they will be predictable, and will perhaps explain why there is some correlation in the success and failure of year classes on the two sides of the Atlantic.

Finally to round out this whole topic, it is necessary to return to the frictional force of the winds on the sea surface. If it is assumed that this force is proportional to the wind velocity, then it seems unlikely that the ocean currents will vary significantly in strength from year to year. It is in low latitudes in the trade wind belts that the currents mainly receive their energy and, as is well known, the trade winds are comparatively steady. How can the strength of the major ocean currents vary in view of the relatively constant strength of the winds in low latitudes?

The recent studies of Rossby have pointed to a possible explanation. He has found evidence that there is a critical velocity below which the winds can get but little grip on the sea surface. If this critical velocity falls within the normal range of the trade winds, then it is clear how in certain years the currents can absorb much more energy than in others, even though the wind variation may be slight. To those who believe that the Gulf Stream is a variable current, this possible mechanism seems most significant. Therefore, it is part of the new cooperative program with the Bermuda laboratory to secure observations that can be used to determine the frictional force exerted by winds of different strength and duration. Likewise, it is planned to investigate the effect which the stability of the surface layer has on the wind current. Only in this way can it be proved whether or not the local winds off the European coast are as important to the climate and fishery as are the variations in the strength of the main circulatory system of the North Atlantic.

(Contribution No. 171 of the Woods Hole Oceanographic Institution.)

(This article is based upon a lecture given at the Marine Biological Laboratory on August 19).

THE COLLECTING NET

Vol. XIII, No. 8 SATURDAY, SEPTEMBER 3, 1938 Annual Subscription, $1.50 Single Copies, 30 Cents.

POSSIBILITY OF ELECTRICALLY AFFECTING DEVELOPMENTAL PATTERN

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There seems for some time to have been a growing feeling among biologists that the arrangement of the parts of an embryo into a uniform and symmetrical whole is based in some manner upon electrical forces existing between cells or groups of cells.

This attitude was crystallised in 1935 by Burr and Northrup in their "electro-dynamic theory of life," in which it is alleged both that a living organism erects an electrical field around itself and that this field controls the pattern of the organism. Such a theory is plausible upon theoretical grounds but it is necessary, before it can be accepted, that we should examine such known electrical effects as might produce the field and further to inquire by experimental means by what mechanism this field could effect the arrangement of cells.

It must be emphasized that we are not dealing with such intracellular (*Continued on page 204*)

M. B. L. Calendar

MONDAY, September 5, 1938, 8:00 P. M.

Lecture:

"Invertebrate Zoology and Oceanography."

Dr. Mary Sears, of the Woods Hole Oceanographic Institution

The Business Office of the Marine Biological Laboratory will be closed on Monday, September 5th.

THE REMAKING OF CHROMOSOMES

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Institute of Animal Genetics, University of Edinburgh

I. The Problem of Structural Change in Chromosomes

It is a biological commonplace that species differ in the number of their chromosomes, and in the shapes and sizes of their chromosomes, and that even individuals of the same species may differ somewhat in these respects. For example, Metz's studies of chromosome configuration in species of Drosophilinae showed differences of all these kinds. Occasionally, in passing from one species to another, a given chromosome would seem to have changed its size; in rare instances it might change its shape; oftener two rod-like chromosomes would seem to have fused into one V, or *vice versa*; and sometimes a chromosome would seem to have disappeared entirely.

Until the last decade, the number and kinds of steps involved in the formation of any of these observed chromosome differences could only be guessed at, for their actual origination had very rarely been

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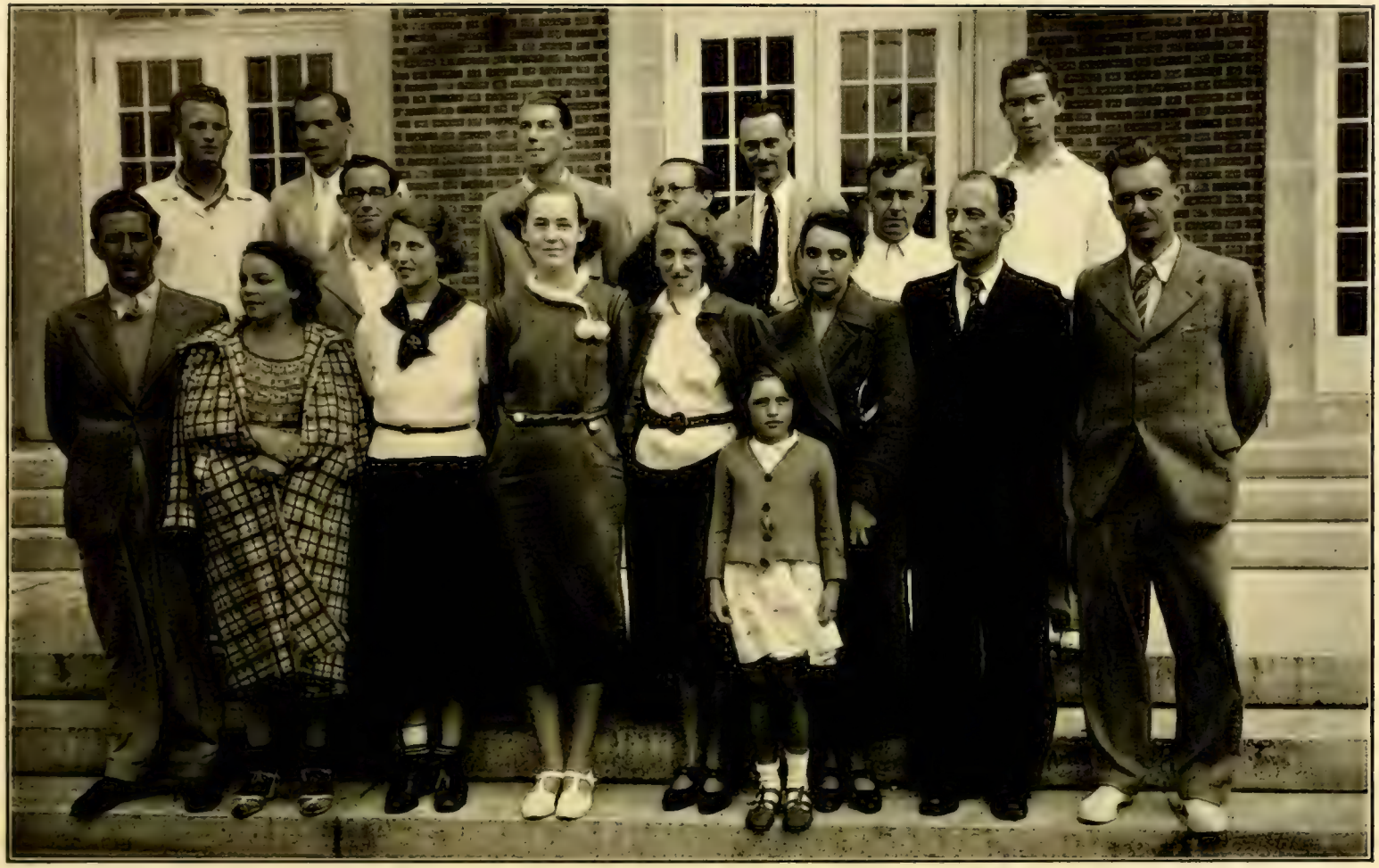

SOME OF THE ROCKEFELLER FOUNDATION FELLOWS AT THE MARINE BIOLOGICAL LABORATORY

Back rows: Helmut Gordon, K. Lissák, J. M. Yoffey, Peter Gray, L. von Bertalanffy, M. J. D. White, Manfred Kiese, H. J. Curtis.

Front row: Stephen Karady, Mrs. Lissák, Mrs. Gray, Sarah C. Bedichek, Mrs. von Bertalanffy, Marjorie S. Newton. Mrs. Grabar, Pierre Grabar, William H. Newton.

observed. Cytology was in fact very vague even about the nature of the observed differences, for the minuter details of chromosome structure were as yet rarely a subject of sufficiently accurate comparative analysis. Thus we could not tell, in the comparison of Drosophila species, whether the shape change, involving as it did a difference in spindle fibre position, consisted in the formation of a new spindle fibre locus, or "centromere" as Darlington calls it, together with the disappearance of the old one, or in the shift of just this locus from one place to another, or in some sort of rearrangement of a larger part of the chromosome, containing the centromere. Again, we could not tell whether the small chromosome had really gone out of existence when it seemed to disappear, or whether it had somehow been swallowed up in some other part of the chromatin complex. And we could not tell, in the change of rods to V or *vice versa,* whether or not the process was unidirectional, whether it involved mere fusion of whole chromosomes, or mere breakage; whether the centromeres themselves fused, or somehow became split, or were formed *de novo,* or whether one of them, or a whole chromosome region containing them, became lost or reduplicated.

A few cases—that had necessarily been imperfectly analyzed—were, to be sure, known, chiefly in Drosophila, which had led to a number of rather crudely mechanical views regarding the nature of chromosome changes. Among these was the belief that chromosomes might undergo simple breakage, perhaps with loss of the fibreless ("acentric") piece or pieces, but with survival of the other one at any rate. This was supposed to have occurred in "Plexate," in Stern's "Y", and in "Pale" translocation. It was also thought that the detached fragments thus formed might sometimes become attached to the ends as well as to the sides of other chromosomes, or of other parts of the same chromosome. Supposed cases in point were Stern's XY, "Pale", and Sturtevant's supposedly terminal inversions. And it was further believed that bodily fusions of one whole chromosome to another one might occur, as in attached X's. On the basis of such interpretations, as well as on Sturtevant's more accurately worked out conception of inversions—which, however, still included the idea of terminally attached inversions, as above noted—Drosophila workers and others attempted to picture the changes of chromosome structure that had occured in evolution, and that were occasionally found distinguishing individuals. In the earlier X-ray work too (1927-1930) the author must plead guilty of attempting to picture the chromosome changes found in largely the same terms.

While, as we shall see, time has shown that these earlier interpretations were wrong in certain features, they were nevertheless right in some very important essentials, and especially in so far as they tried to make their picture conform to the principle long ago laid down by McClung, chiefly on the basis of his comprehensive studies in Orthoptera, "that numerical variations involve only rearrangements of the persistent chromatin aggregate" (cited from McClung, 1921). One important principle that is included in this general idea of the continuity of the component parts as such, is McClung's concept of the persistence of the centromere, or achromite as he terms it, and of the necessity of the latter for chromosome persistence. But the work of the past decade has made it possible to extend this principle of the preservation of the individuality of chromosome parts in some rather unexpected ways, so as to bring under one general scheme all the different types of alteration of chromosomes that had been separately dealt with in the earlier work under the various special conceptions above noted.

These more recent advances in our knowledge of chromosome evolution have come about through the convergence of a number of lines of investigation. For one thing, the X-ray technique has provided a wealth of material illustrating the origination of multitudes of chromosomal changes, of diverse sorts. In Drosophila, the more exact analysis of these findings has followed, on the one hand, from the elaboration of more refined genetic methods and, on the other hand, from the amazing technique of observation of the chromosomes in the salivary glands inaugurated by Painter. Among the cases so investigated there have been enough of spontaneous origin (including intraspecific differences found in nature) to show that the same principles apply to them as to the products of irradiation. Meanwhile, too, the improvement of cytogenetic method and interpretation in plant material, due especially to the work of Stadler and McClintock and of Darlington and his school, following upon the earlier advances by Belling and Blakeslee, have led to a series of findings in maize, Tradescentia, Datura and other plant forms that are in some essential ways similar

THE COLLECTING NET was entered as second-class matter July 11, 1935, at the Post Office at Woods Hole, Mass., under the Act of March 3, 1879, and was re-entered on July 23, 1938. It is devoted to the scientific work at marine biological laboratories. It is published weekly for eight weeks between July 1 and September 15 from Woods Hole, and is printed at The Darwin Press, New Bedford, Mass. Its editorial offices are situated on Main Street, Woods Hole, Mass. Between June 1 and October 1 communications should be addressed to Woods Hole, Mass.; at other times they should be directed to THE COLLECTING NET, Garrison, N. Y. Single copies, 30c; subscription, $1.50.

to those of the Drosophila work. But as there still remain some important differences, susceptible of alternative interpretations, I shall base my present discussion mainly upon the Drosophila findings.

If we should pass in review, in detail, all the findings concerning chromosome rearrangements which have been made in Drosophila in the past decade, we should see that all of them unite in supporting the proposition that structural changes in chromosomes consist, in general, not of simple breakages or fusions, by themselves, but of *exchanges* in the linear attachments of sections of chromosomes. That is, as in crossing over, a section of chromatin becomes disconnected at some point from the section to which it previously was attached, and, connected, at the same point, to a point on another section at which the latter likewise has become (or becomes) disconnected from its previous association. Thus at least two breakages are necessary, and, as in crossing over, there must be reattachment, and this must occur between the broken ends, not between originally free ends, or between a broken and a free end, or between any end and the side of a chromosome giving a branched structure). Unlike what occurs in crossing over, however, the two points of interchange are not homologous, and the process has therefore been referred to as "illegitimate crossing over", a term used by both Darlington (1932) and the author (1932) independently. (Whether or not the process involves touching or close juxtaposition of the parts broken, previous to their breakage, as in crossing over, is a matter which we shall consider later). Since the time when the author put forward this principle of exchange as a general interpretation of structural changes in chromosomes (at the Genetics Congress, 1932), the evidence for it, in Drosophila at least, has become far stronger, and the various types of apparent exceptions to it there have received their explanations in accordance with it, especially when it is considered in connection with the interesting facts concerning the so-called "inert" or chromocentral regions which have come to light in recent years.

II. Special Properties of the So-Called "Inert Regions."

The realization that there were special regions of the chromosome complex in which, for a given length of chromosome as seen at mitosis, there were relatively few active genes, goes back to the evidence, to which Metz and I long ago called attention (see Muller, 1914), that the Y chromosome of Drosophila, though apparently large, acts as though virtually "empty."* The cause of the apparent emptiness was only in part understood in 1914, however, as it was not realized that any other factor entered in than that of degeneration by mutation pressure under the influence of permanent heterozygosity. Much later, Painter and I (1932) unexpectedly found that the X chromosome has a homologous "inert region", of similar apparent emptiness, and both Heitz and we noted indications that the other chromosomes might have similar regions in the neighborhood of their centromeres.

Our findings on deleted X-chromosomes (1929, 1932), and on translocated autosomes and Y chromosomes, and Oliver's results on inversions of the X-chromosomes, led us, further, to the conclusion that breakage occurred in these "inert regions" with a frequency as great, for a given length of chromosome as seen at mitosis, as in the so-called "active" regions. This high breakability has also been found in the studies of Patterson et al (1934) on translocations. It was for this reason that we were at first inclined to regard the actual chromonema in the "inert regions" as being as long, for a given length of mitotic chromosome, as that in active regions.

However, in a review of studies carried on in collaboration with Painter and with Gershenson, it appeared (Muller and Gershenson, 1935) that these breakages in the "inert region" had occurred only at two or three specific points, so far as the chromosome as seen at mitosis was concerned. That is, the regions between these points must be regarded as large genetically indivisible "blocks," produced by individual genes, and so the number of genes and the actual length of breakable chromonema outside the blocks in these so-called "inert regions" must be far smaller for a given length of mitotic chromosome, than in active regions. This was in itself a clue to their apparent inertness. But, this being the case, within a given length of this chromonema, or for a given number of genes outside of the "blocks," breakage must occur with far greater frequency in the "inert" than in the "active" regions of the chromosomes. The above conclusions in turn agreed with and explained the finding of Painter (1933, '34) that in the salivary gland chromosome the "inert regions" were apparently absent, modified by the later finding by Prokofyeva and myself (1935) that they were indeed represented,

* Until shortly before that, Drosophila geneticists, accepting an early surmise of Stevens which she had later corrected, had usually assumed that Drosophila had the XO type of sex determination and that what we now call the Y was an autosome with the X attached, but Metz's comparative studies of male and female and Bridges' studies of non-disjunction had later (1913) proved that this chromosome was really a Y. In this was the above conclusion was made possible.

but only by very short regions, containing about one or two dozen faint bands (and hence probably somewhat more genes) located within and near the chromocenter.

In fact, the chromocenter itself consists, as Prokofyeva first showed (1935), merely of these parts of the chromosomes clumped together, and it has a structure essentially like that of the rest of the chromosomes. It had been far more difficult than the rest to disentangle only because of the fact that these regions of all the chromosomes tend to conjugate into one mass and because in these regions, as a secondary consequence, the disc structure is less clearly arranged and there is a lesser concentration of nucleic acid. This conjugation of these regions of different chromosomes indicated an at least partial homology between them, and further evidence for this was obtained in the finding of occasional crossing over between the X and both arms of the Y chromosome, when present in the female (Phillip, 1934; Neuhaus, 1936), and in the presence of bobbed in both arms of the Y as well as in the X (Neuhaus). Especially pertinent in this connection is Gershenson's recent finding (1936, unpublished) that the chromocentral region of the X, as exemplified by an X chromosome with the "active" portion deleted, tends to segregate from the single fourth chromosome in the meiosis of "haplofourth" individuals.

It is tempting to think that this homology is caused, in part at least, by a common derivation of the regions near the centromeres of different chromosomes, a derivation not inordinantly remote in time because of the occurrence, in evolution, of transplantations of whole chromosome arms from one of these regions to another, as explained below. At any rate, there is evidence that the genes which do exist in such a chromosome region of any individual chromosome pair are on the whole more dispensible than those in ordinary regions, i.e. their loss, as well as their duplication, causes less functional disturbance, just as would be expected if their functions were being in part subserved by similar genes in the corresponding regions of other chromosomes. But, whatever the explanation, it seems clear that this fact of their comparative dispensibility is of importance in the alteration of the chromosome number in evolution.

The chromosome regions in question are thus seen to be peculiar not only in respect to their including a centromere but also in a number of other respects. Chief among these are the following. (1) Their inclusion of the large "blocks" seen at metaphase, rich in nucleic acid, and each dependent only on one gene. (The function of the blocks is as yet unknown, but Gershenson's studies indicate their relative unimportance in segregation.) (2) The mutual and multiple attraction of these genes for one another, no matter what chromosome they lie in, with resultant formation, in resting stages, of a "chromocenter," with its various morphological and physiological peculiarities. (3) The high tendency of these regions to breakage includes, as we (Muller, Belgovsky and Raffel, 1936, '37) have recently found, a tendency to two or more breaks near to one another, with minute rearrangement, as well as to gross rearrangement. Moreover, it extends to neighboring regions, that have, through structural rearrangement, come to lie near the originally chromocentral ones. This tendency to breakage is probably a resultant of the other peculiarities caused by the multiple attraction. (4) Their relative dispensibility in cell functioning, above discussed. (5) Their specific effect (see Offermann and Muller, 1931, 1932) in reducing the frequency of crossing over in regions near them, an effect extending for considerable distances and gradually fading off. This effect is especially responsive to differences in cellular conditions caused by temperature, irradiation, aging, etc. It, like the tendency to breakage, is doubtless, in part at least, a resultant of the multiple attractions, which would tend to conflict with one another. (6) An unusually marked, and peculiarly variable (mosaic) position-effect on the functioning of genes lying in and near these regions, which will be discussed subsequently. This also is probably to be attributed to the multiple attractions.

The various effects mentioned—on synapsis and chromosome morphology, on breakage, on crossing over, and the position effect—are not due directly or solely to the inclusion of a centromere, or of the chromatin "blocks", or both. The latter elements, together, appear to comprise only about three main genes, whereas genetic studies have shown the existence of at least several other genes in this region of the X-chromosome. These include genes for fertility, similar to those in the Y, as found by Dubinin, and genes affecting the various properties above mentioned. And the cytogenetic studies of Prokofyeva and myself (1935) taken in combination with the observations of Bridges (1938) show this region to contain some two dozen faint bands. Judging by our work on the correlation between genes and bands, there are at least as many as and probably considerably more genes than visible bands. Now our studies of individuals in which, as a result of irradiation, the chromocentral region of the X-chromosome had been broken at one place or another and transplanted elsewhere, show that even those parts of the region which not not contain a centromere, or do not contain a chromatin block, and which have been removed from their propinquity with these parts, still possess

the peculiar properties above mentioned. This was proved for the multiple attraction and associated peculiarities of cytological structure by Prokofyeva in 1935, and independently observed by Schultz (1935); for the breakage tendency by Belgovsky and myself (working with scute 8, scute S1 and Bar M2) and for the crossing-over effects by Offermann (using flies homozygous for the inversions scute 8 and scute 4). Numerous of our cases prove that the same principle holds for the peculiar position-effect before mentioned. Thus these various characteristics pertain to genes scattered throughout the chromocentral region, not merely to the centromeres or blocks, and they may be expected to occur even in regions not containing the latter, if these regions manifest the multiple attraction and the associated peculiar cytological features of chromocentral regions as observed in salivary glands. We shall see that this conclusion is significant in a number of ways.

A number of investigators had noticed some tendency of the distal ends of the chromosomes to lie touching one another; and this was even more pronounced in Chironomus and some other species than in Drosophila. And, in the case of the small fourth chromosome of *Drosophila melanogaster*, there was a tendency for it to curve about and come into contact with the chromocenter (Bridges, 1935). Studying the end regions more minutely, Prokofyeva (1937) has found that the unions in question are not merely end-to-end. There are definite terminal regions on all of the chromosomes, usually consisting of several faint bands that morphologically resemble those in the neighborhood of the centromeres. And these regions of different, non-homologous chromosomes have a tendency to conjugate with each other, just as do the regions near the centromeres, though not as marked a tendency, perhaps because these terminal regions are considerably smaller. They have a tendency also to conjugate with these chromocentral regions that lie in the neighborhood of the centromere, though this tendency is not apt to be expressed except when, as in very short chromosomes like the fourth of *D. melanogaster*, these ends naturally lie near the chromocenter. When, through translocation, other ends than that of the fourth are brought similarly near the chromocenter (as in the case of the left end of the X in our "mottled-5" translocation), they too tend to conjugate with the latter in fairly typical chromocentral fashion.

The above observations agree with genetic evidence which shows that the terminal regions, in some of the chromosomes of *D. melanogaster* at least, have (1) a relatively low frequency of crossing over (at least in the second and the X, though that in the latter probably depends in part at least on the "bulb" region, which Prokofyeva regards as semi-chromocentral), (2) a relatively high frequency of breakage (at least in the second and the X, and especially the former, as shown in studies of Muller, Koerner and Vogt, and of Patterson, *et al.*, (3) a relatively high dispensibility, as shown by the viability of individuals having deficiencies in these regions, (at least in cases involving both ends of the X, and the right end of the fourth chromosome, studied by L. V. Morgan, Demerec, Catcheside and Beadle, Prokofyeva, and myself), and (4) the peculiar mosaic position-effect (at least in the third chromosome) found otherwise only in the case of regions about the centromere. All this leads us to agree with Prokofyeva that these terminal regions are similar in their properties to those normally located near the centromeres, and that, like the latter, they may be termed "chromocentral", i.e. possessed of multiple conjugating properties, indicative of a mutual homology of some degree, with its various consequences.*

In view of the fact that the chromocentral regions in the neighborhood of the centromere, i.e., the centromeric regions, contain some dozen or dozens of bands and of genes, and the gene (or genes) for the centromere itself has not yet been identified in the salivary chromosome (though its region has been somewhat narrowed down), there seems no sound reason for maintaining that it is actually the very terminal gene, even in the case of rod-like chromosomes such as the X and the fourth in *D. melanogaster*. As for their appearance at metaphase, we could hardly expect the centromere, being so near the end of the salivary chromosome work shows it to be, to result in a chromosome distinctly different from a rod, as seen at mitosis. In fact, however, the minute observations of mitotic chromosomes carried out by Prokofyeva and by Heitz have strongly suggested a non-terminal, that is, a sub-terminal, position for it. And agreeing with this is Prokofyeva's observation of small "heads", as she calls them, on the right distal ends of both the X and the fourth chromosomes in the salivary glands. These "heads" resemble somewhat the left distal ends, being not as thoroughly chromocentral as the region slightly to the left of them, which she supposes to contain the centromere itself.

This conception of the existence of another terminal chromocentral region, beyond the centromere of the apparent rods, and having the high breakability characteristic of other chromocentral regions, also affords a ready explanation, consistent with the other known facts regarding chromo-

* But whether their liability to exchange with one another is primarily a cause or effect of this homology, or both, is a question that cannot yet be answered with assurance.

some rearrangements, for the frequency with which, in translocations, sections of other chromosomes can be attached beyond the centromere of the X and fourth chromosome, to give rise to chromosomes that are obviously J or V shaped. Thus Panshin, in 1937, and Stone, in 1938, have both found "mottled" fragments, from the left end of the X attached to the right of the centromere of the fourth chromosome, to give tiny medially attached chromosomes, both arms of which were approximately the size of the normal fourth. Similar attachments of pieces of other chromosomes to the right of the centromere of the X, forming a J or V shape chromosome, were reported by Muller and Painter in 1929 and have since been seen frequently. Evidently what happened in such cases was the breaking off of the dispensible terminal region lying to the right of the centromere, and its replacement by the larger section from the other chromosome, so as to give a structure which now is obviously non-terminally attached, and which bears witness to the originally more cryptic sub-terminal nature of the attachment in the so-called "rod" chromosome. A rigorous proof of this point awaits a more exact salivary analysis of the chromocentral regions in question in the translocated chromosomes, as compared with the normal ones. Meanwhile, however, the cases certainly do not stand opposed to our general conception of chromosome structure and of chromosome alteration.

III. Types of Conformation of Chromosome Rearrangements.

Despite the differences above noted between the properties of the chromocentral and free regions of the chromosomes, there is no reason to believe that they differ in regard to those fundamental features of chromosome make-up which determine the mechanism whereby structural rearrangements take place. That is, both regions undergo structural change by means of *exchanges* of linear connections at given points, involving both breakage and reattachment, only these exchanges occur more readily, or at least more frequently, in the chromocentral regions than elsewhere. And, as we shall see, the differences between the regions do affect to some extent the subsequent fate of the reconstituted chromosomes.

Accepting, for purposes of exposition at least, this principle of exchange, let us now see what different kinds of alterations it will result in, according to the topographical accidents of how many points of breakage there are, where in the chromatin they lie, and just which broken ends become attached to one another. We may consider first the situation in the simplest possible cases, that is, when there are only two points of breakage. The first case we may examine here is that in which these two points of breakage lie in the same chromosome. In this particular case, if the broken ends of the two distal sections each unite with that broken end of the interstitial section which was originally attached to the other distal section, an *inversion* results. If the breaks were to one side of the centromere, the inversion may be termed *"paracentric"*, and it will be noted that the proportions of the two arms, and hence the general shape of the chromosome as seen at mitosis, is not changed. But if the breaks included the centromere between them, being *"pericentric"*, the mitotic chromosome will have the relative sizes of its two arms altered, except in the special case in which the two distal sections are sensibly equal in size. The union of the broken ends of the two distal sections with one another results in the formation of a *deleted* chromosome, while the two ends of the interstitial section unite together to form a ring.* In the case of paracentric breaks the ring includes no centromeres and hence cannot be transported at mitosis and is eventually lost, while in the case of pericentric breaks it is the other fragment which is "acentric", and becomes lost, while the ring has a centromere (being "monocentric") and may persist for a time. Rings tend to be lost eventually, however, because of the abnormal chromosome types that, for purely mechanical reasons, they give rise to at crossing over (see L. V. Morgan, 1932) and, in some plants, also at mitosis (McClintock, 1933).

The breaks may be near together, so that the inversion or deletion formed is minute. In fact, a curve showing the frequencies of breaks different distances apart, in relation to the frequencies to be expected on a random distribution of breaks, would undoubtedly show a considerable peak for breaks that were close together—a fact the cause of which we shall inquire into later.

As a kind of opposite to the cases of minute rearrangement we have another, also disproportionately abundant class of cases, in which one of the breaks occurred in a chromocentral region, say, that next to the centromere—and the other break occurred either in a so-called "active" region (better to say, "free" region) or in another chromocentral region—say, that at the end of a chromosome. Here then there is a tendency for the included interstitial sections to be larger than expected on a random distribution of breaks, and not infrequently nearly the whole arm of a V-

* We are omitting from consideration the cases—presumably numerous—in which broken ends that were previously united with one another reunite to reconstitute the original type of chromosome, and also those hypothetical cases—perhaps numerous but, if so, not viable—in which the broken ends fail to undergo reattachment.

shaped chromosome, or nearly the whole of a rod-like chromosome becomes deleted or inverted.

Continuing our perusal of types of chromosome rearrangement, we may next consider what happens when the two breaks occur in different, non-homologous chromosomes. The process is mutual translocation, and the resultants can survive in the case in which those fragments have united which are complementary with respect to possession of a centromere, but not in other cases, in which only acentric and dicentric parts are formed.* Since breakage occurs oftener in chromocentral regions, especially centromeric ones, there are not infrequently exchanges of whole arms between V-shaped chromosomes, and exchanges between one arm of a V and the main arm (that is, virtually the whole) of a rod-shaped chromosome. There are also exchanges between two rods, resulting in one large V and one minute chromocentral V, the loss of which latter could probably be tolerated without difficulty.

Any change involving an increase of chromosome number, such as the change of a V to two rods, is more complicated, except where the Y chromosome serves to supply the extra centromere. For in other cases a disadvantageous amount of duplication of other regions would be entailed unless, secondarily, they were removed by deletion. For this reason the Y has probably served more often as "anchorage" to various chromosomes, in their process of origination as independently attached chromosomes (as proposed by Muller and Painter, 1932). This situation would of course work towards an increase in the homology of the chromocentral regions of different chromosomes.

There is also the equivalent of mutual translocation, occurring between homologous or sister chromosomes. The result here, when viable at all, is usually the formation of a deficiency, equivalent to a deletion, in one reconstituted chromosome, and of a duplication, or as Bridges aptly terms it, a "repeat", in the other one. The duplicated section immediately follows its prototype, and the two sections have their genes lying in the same direction, as in the well-known bar-eye duplication. I am provisionally terming this type of repeat a "joined tandem." Unless they are rather small, duplications, by causing an "overdose" of some genes in relation to others, give individuals with low or even zero survival powers, especially when they are homozygous. Small duplications, however, becoming established in a species, can eventually increase its evolutionary potentialities by providing it with more genes which, though at first alike, may become different through mutation. This is in fact *the* method of increase in gene number.

When, as not very infrequently happens even in spontaneous rearrangements, three breaks occur at once, the possibilities are considerably more numerous. Three breaks in the same chromosome, in addition to giving us obvious combinations of the changes obtained from two breaks, such as two adjoining inversions, or deletion and inversion, also give us another type. In this a section, large or small, has, as it were, been excised or deleted from one place, the broken ends on either side of which are joined together again, and inserted into another position, either in the original or opposite direction.

When two of the breaks are in one chromosome and the third in another chromosome, a piece may be deleted by the two breaks and inserted into the space made by the break in the other chromosome, thus constituting an "insertional translocation." Here the translocation of material is not mutual, but unidirectional, purely from the former chromosome to the latter. As opposite extremes of proportions for such cases we may mention the case of scute 19, in which a piece consisting of only about 4 genes became deleted from the X chromosome and inverted into a long autosome (the second), and on the other hand, translocation X-1VI (or "W 13"), in which a piece comprising about half the X, and cut off, on its right end, within the chromocentral regions, became inserted into the tiny fourth chromosome. Other modes of reattachment, following three breaks, of which two are in one chromosome, can give mutual translocations, in which there is an inversion or deletion adjoining the point of chromosome exchange.

When the two chromosomes engaging in triple-break rearrangements are homologous the piece which was inserted constitutes a repetition of some part already in the recipient chromosome, and so it results in the presence of a "repeat", either (1) shifted away from the original position of such a section, or (2) adjoining the latter if two of the breaks in the homologues were at identical points (owing perhaps to a chromosome

* No doubt one reason for the loss of dicentric chromosomes is that during mitosis they would sometimes be pulled in both opposite directions at once, and hence would move in neither direction. If this were their only disadvantage, it might be that they could live in cases in which the two centromeres were close together, in which event the normal chromosome should sometimes have two or more neighboring loci that served for fibre attachment. There is as yet on evidence to indicate that this situation may obtain in Drosophila, but we are at present testing the matter out more fully. On the other hand, in plants, multiple juxtaposed centromeres have been described, and McClintock has very recently (1938) presented cytogenetic evidence of them, since each of the two sections formed from a breakage in the appropriate regions received a functional centromere. If this can happen in Drosophila, however, the occurrence must be vanishingly rare.

breaking before its division), or (3) within the latter. The two like sections may then be either in inverted position with respect to each other, forming a "reflexion", or facing in the same direction, forming a "tandem."

Finally if the three breaks are all in different chromosomes, if no two broken ends that were formerly attached together rejoin as before, and if the fragments that unite are in each case complementary with respect to possession of a centromere, a type which may be termed a "triple mutual translocation" results.

Virtually all of the viable types of two-break as well as three-break rearrangements above referred to have been found. As for the different types of more complicated rearrangements producible with more than three breaks, it would take us too far afield to follow them through here. But some of these too have been found, as, for example, Stone's six-break "translocation 12", in which, for one thing, the life of an acentric ring, formed by deletion, was saved by having it broken at another point than that at which it had become joined together, and having these broken ends serve for its insertion, as a straight piece, into the space formed by the break of another chromosome (the fourth). At the same time this ring had a minute piece (containing the locus of "cut ring") deleted from out of it, at still another place, and inserted into still another chromosome (the second). Nor should we take time here to note the various "aneuploid" types, that is, types having excesses or deficiencies of chromosome sections, or both, formed secondarily in later generations by crossing over or chromosome recombination occurring in germ cells heterozygous for one or more rearrangements. As before noted, such individuals with excesses, and especially those with deficiencies of genes, tend to be inviable, especially when homozygous, except in special cases.

In presenting the evidence, six years ago, for the proposition that structural changes in chromosomes, in Drosophila at any rate, were, in general, caused by exchanges, conforming to the principles mentioned above, I had to make the qualification that a small number of cases did exist which seemed to disobey these principles. The cases then outstanding have now all been satisfactorily explained. Some (like 19) then thought to be terminal translocations have been found to be insertions. So have the supposed cases of attachment to the side of a chromosome, outstanding among which was "Pale translocation." Other cases, apparently terminal translocations or inversions, have been found to involve breaks near the end. The formation of attached X's, as well as their detachment, are seen not to be mere fusions of rods into a V and *vice versa,* but cases of crossing over with a Y chromosome. Ring chromosome formation is found to involve the loss of a minute amount of terminal chromatin, thus enabling the sticking together to occur. And the apparent cases of simple breakage, with loss of the terminal fragment of certain special chromosomes, have recently been shown by Belgovsky, Prokofyeva, Raffel and myself to represent minute rearrangement of the chromocentral region, of which a considerable amount exists here in a subterminal position. A paper of Raffel's at the present meeting gives further evidence of this.

Meanwhile, a very few new cases have appeared, that have been interpreted as terminal deficiencies, not involving reattachment of other chromatin at the breakage points, and certain others that have been interpreted as simple fusion of unbroken rod-shaped chromosomes, at their proximal, that is, centromere-bearing ends. But, considering all the deficiencies examined, it is to be expected that two or three of them would have involved distal breaks in the terminal chromocentral regions, so close to the end that the presence in them of the terminal gene could not be demonstrated. For, as Prokofyeva and I have shown, single genes are at or below the limit of vision, particularly in such regions, and breakage is especially frequent there. On the other hand, an active search which we conducted for such cases failed to yield any, while giving numerous cases of obvious exchange of connections. And as for the apparent fusions of rods, they fit very well into the scheme of mutual translocation, involving breakage in the centromeric chromocentral region of both chromosomes, the breakage in one chromosome being at the base of the long arm, and in the other in the rudimentary arm. The minute size of the latter, in the mitotic chromosome, is no objection here, in view of the known high tendency to breakage of minute regions of this kind.

We cannot, of course, rigorously prove the universal negative, that no cases contrary to our principles occur. But, in view of the considerations given, and our experience with the earlier cases that challenged us, it seems better, as William of Occam would have agreed, not to involve further principles to account for structural chromosome changes, until really cogent evidence has appeared for the inadequacy of those already found.

IV. On the Mechanism of Rearrangement

What is the order of events when the exchange between non-homologous chromosome parts takes place? Nine years ago two general possibilities with regard to the mechanism of chromosome change had presented themselves that seemed to be alternative (Painter and Muller, 1929). Two chromosome parts that were to undergo rear-

rangement with one another might come into contact, perhaps even fuse first, at the points where they were to undergo breakage, and then break, the breaks thus having a common cause, as must be true in crossing over. Or they might break first, perhaps independently of one another, and the broken ends, being somehow adhesive, might later find and fuse with the parts which they were to be attached to. Six years ago Stadler advocated the latter view but I opposed it, in favor of the former one, on the ground that the rearrangements produced by radiation were not proportional to the square of the dosage of the latter, as they should be if they were the product of two independent breakages, the chance of each of which, singly, was proportional to the dosage itself. Today, for several reasons, I believe that there was probably truth in both views.

It is well known, since the work of Oliver, Hanson, Timoféef-Resovsky, and many others, that gene mutations vary in frequency directly with the dosage of radiation, that is, with the number of ions formed, regardless of the time distribution of these ions of their type of distribution in space (as determined by the hardness of the rays). And this furnishes good evidence for the conclusion that the individual mutations are caused by individual ionizations, that happened to be favorably situated. If rearrangements of chromosome sections followed the same law, a single rearrangement, with its two or more breaks, would likewise have to be judged to depend on one favorable ionization, that acted as common cause to both breaks, and so the breaks would have been somehow connected. This could hardly have occurred unless both points of breakage had at the time lain near together. But, as above mentioned, if the breaks were independent, each depending on at least one separate ionization, the coincidence of two breaks, necessary to allow an exchange of the broken ends, would vary in frequency as the square of the dosage.

The indications from the earlier work (especially Oliver's in 1930 and '32) were that rearrangements did not vary in frequency as markedly as the square of the dosage; hence the conclusion which I first drew. Yet Oliver's results did suggest a higher rate of variation of frequency than that of the dosage itself (i.e., than its first power), so further experiments were called for. The first of these, carried out in Berlin in 1933, are presented herewith. It will be seen that these two-break deletions vary in frequency at a rate greater than the first power of the dosage, and yet less than the square. This seemed hard to believe, but Belgovsky in Moscow has tested out the same question on an extensive scale, using translocations, and has consistently gotten the same result. For the doses used, the rearrangements vary in frequency about as the 3/2 power of the dosage. And Berg, Panshin and Borisoff, in Leningrad, have found the same thing for inversions. Confirmations have now been obtained by Dubinin's co-workers also.

Of the various possible explanations of this peculiar result, I incline to the view that some of the two-break rearrangements are the product of two separate breakages, caused by two separate ionizations—in these cases the broken ends must find and fuse with each other later, and that others—a comparable number—depend on two breakages both of which resulted, secondarily, from the same original ionization. Where is Occam's razor now, it may be asked. The answer is that there are further reasons in support of the existence of both of the processes postulated.

In support of Stadler's thesis that breakage may occur without contact of the parts concerned in reunion, it is now possible to apply the result of a test which I proposed in 1932, as to the mode of exchange of the broken ends. If, as in crossing over, chromosome threads make cross-connections only with the parts which they have been touching, or nearly touching, at the time of breakage, then if the broken end which we shall call A_1, lying to the left of a certain breakage point, A, becomes joined with the broken end which we shall call B_2, lying to the right of breakage point B, the segments of thread at which these breakages occurred must have been in contact here, and hence the broken end, A_2, lying to the right of breakage point A, can undergo union only with B_1, which lies to the left of breakage point B. That is, the relation must be a reciprocal one. A_2 cannot, in that case, unite with a different broken end, call it C_1, that was formed by a third breakage, unless we suppose that the third piece of chromosome thread also had touched the other two, and not only touched them, but touched them at exactly the point where they themselves touched one another. This requires a precise three way cross.

Although Offermann has, to be sure, suggested that such a triple contact might be produced by the mechanical process of the third thread sliding along until it came to rest in the notch of the other two, it would seem far-fetched to explain many cases in this fashion. And all the varied types of triple-break rearrangements—of which not a few have been found—would require this same explanation. Moreover, Dubinin and his co-workers have found that even in rearrangements derived from more than three breakages the type of exchange is apt to be such as would require, on the contract-breakage scheme, the meeting of four or more threads at exactly the same point. Hence he has given up this hypothesis of accounting for rearrangement, which he had earlier championed.

TABLE I.

Relation of Frequency of Deletions to Dosage of X-rays

(Data of Muller, Koerner and M. Vogt, 1933)

Cross 1: Rayed sc^8 Bw^a ♂ ♂ X yw ♀ ♀ (X's separate).
Cross 2: " " " ywf " (X's attached).

Exp. No.	Cross	Dose in r (Rel. value accurate within same experiment)	Total Offspring from Y-bearing Sperm	Offspring from X-bearing sperm carrying			Large deletions "expected" for higher doses
				no detectible alteration	misc. abberations	large deletions found	
1		4000	10,624½	10,989	38½	24	18
to	1	2000	19,109½	18,503½	25	16	
3		control	16,469	17,330	2	1	
		4000	2,134	1,529	4	27	17½
4	2	2000	3,642	2,969	2½	17	
		control	3,257	3,040	0	2	
5		6000	2,629	3,642	9	42	26½
&	2	1500	13,132	15,469	5	33	
6		control	6,949	7,408	0	0	
Summary	For higher doses.	4000-6000	15,387½	16,160	51½	93*	62*
	For lower doses.	1500-2000	35,874½	37,941½	32½	66	
	Controls		26,675	29,478	2	1	
(total flies: 161,752½)			77,937	83,579½	86	160	

* Difference = approx. 3½ times its probable error.

(Fractional figures are derived from mosaics)

("Expectation" for higher dose calculated from results for lower dose on basis of frequency being directly proportional to dose in each exper.)

The independence (or comparative independence?) of the frequency of rearrangement in relation to the time over which the total treatment is protracted, in mature sperm—a point on which we are now engaged in obtaining better evidence—likewise argues for the breakage-first scheme. For it is difficult plausibly to explain how the effects of a past ionization can be stored up for weeks, so as to influence the effect of a later ionization (as the 3/2 power rule implies in such cases), unless we suppose that each breakage, occurring at the time of the given ionization that produces it, is saved up as such until, after fertilization, the chromosomes, now in a different state, have a chance to undergo union at their broken ends.

In plants, at least, more direct evidence has been obtained by McClintock and Beadle in the finding that, when a ring chromosome becomes broken, by the opposed pulling of spindle fibres, its parts tend to reunite again at their broken ends, and Darlington has observed reunion of fragments of dicentric inverted chromosomes that were broken at meiosis. (Unfortunately for our tests, these causes do not lead to breakage in Drosophila). Moreover, direct observation of X-rayed cells in various organisms has shown that fragments are formed by breakage induced by the radiation; these would furnish material for the process in question.

Provisionally at least, then, we may adopt the "breakage first" conception as at least one mechanism of exchange. Further tests of it can and

should be made, however. For instance, if it applies to a part of the rearrangements and not all, the curve of their frequency will more nearly approximate the first power of the dosage at low dosages and the second power at high dosages. Moreover, in that case, multiple-break rearrangements should bear certain characteristic frequency relations to one another and to two-break rearrangements, at different dosages. The latter is a matter which we are now endeavoring to test.

If we admit that some at least of the breakages occur before contact of the threads, there are probably cases in which the broken ends fail ever to find one another before the acentric fragment becomes lost at the ensuing mitosis. In that case the centric portion would have the opportunity to continue as a fragment, formed by simple breakage, and without having received a complementary fragment, by exchange, in substitution for the one it had lost. But, as we have seen, there is no sound evidence for the occurrence of such products of simple breakage among the descendants of irradiated individuals. Hence, if we accept the breakage-first hypothesis, we must infer that fragments, even though provided with a centromere, die if their ends are broken ends, i.e., if their ends do not consist of natural termini, derived from the same or another chromosome.

The conclusion can therefore be drawn, if the breakage-first hypothesis is valid, that the terminal gene must have a special function, that of sealing the end of the chromosome, so to speak, and that for some reason a chromosome cannot persist indefinitely without having its ends thus "sealed". This gene may accordingly be distinguished by a special term, the "telomere" (applied by myself and Darlington, and by Haldane, independently). The telomere is unipolar, being attached to only one other gene—that on the "proximal" side of it, and it is thus distinguished from all the other bipolar genes, which are attached to two genes, one on each side. Bipolar genes, then, cannot be made into properly functional unipolar ones by the simple expedient of tearing them loose from their connections on one side. This idea is, it will be seen, merely an extension of the more general one of McClung previously referred to, of the persistence of the individuality, and of the peculiar properties, of the different separable components of the chromatin aggregate, even in the face of extensive rearrangements of these parts.

It may now be asked, why do we still cling to the contact-breakage scheme of rearrangement at all? The answer is, first, because the frequency-dosage relationship for rearrangements, after all, does not follow the typical square rule, and so necessitates some kind of modification of the scheme of merely independent breaks (or reunions). Second, it seems—though this point has not been rigorously proved—as though there were relatively too many rearrangements, such as small deficiencies, which involve pairs of breaks very close together, than can well be accounted for on the idea that all breaks occur independently of one another, even though we supplement this with the very probable postulate that when the breaks have occurred near together they are more likely to undergo an exchanged attachment to one another than when they were far apart. Third, and most important, there is evidence that pairs of breaks very close together obey a rule for the relation of their frequency to dosage which is the same as that of the so-called "gene mutations." That is, their frequency seems to be simply proportional to (the first power of) the dosage. This indicates that the occurrence of each such minute rearrangement, including *both* its breaks, is a result of but *one* effective ionization. For a large proportion of the lethal changes produced by radiation which were formerly regarded as gene mutations are, as shown by the results of Mackensen and Painter, of Demerec and others, really small deficiencies, that is, minute deletions, and so, since lethals of all kinds, taken as a whole, vary in frequency simply as the dosage of irradiation, these small deletions almost certainly obey this rule too. We are, however, now attempting to get more direct evidence on this point.

As a beginning in our attack on the latter point, it was decided to test the relation of frequency to dosage in the case of minute rearrangements affecting certain known genes near the chromocentral region of the special rearranged chromosome scute 8, which we had found to be favorable for the purpose in that the rearrangements in question could be detected by mere inspection of the flies. The results, recently obtained on a considerable scale by Belgovsky, show clearly that these minute rearrangements do in fact obey the rule of simple proportionality of their frequency to dosage, just like orthodox gene mutations. And cytological analyses by Prokofyeva as well as genetic analyses by Raffel, the latter to be reported at the present meeting, show that the changes in question are really minute rearrangements involving exchanges of the connections of two or more breaks, being neither ordinary mutations nor, as first thought, simple breakages with loss of the terminal fragment.

The conclusion thus follows that in the case of most pairs of nearby breaks, productive of minute rearrangements, a single electron-hit somehow results in both breaks. If we try to keep our conception of the mechanism of breakage as nearly alike as possible for both minute and gross rearrangements, we will not suppose that it is the contact *per se* which somehow causes the break in the case of the minute rearrangements. Neither,

as I have formerly shown (1932), can we suppose that a single electron happens directly to strike both nearby points, breaking both; this is precluded on physical and mathematical grounds. We are thus brought back to what I may now nickname as the "bomb" hypothesis, which I proposed when discussing this question in 1932. That is, an ionization that works, so as to break a chromosome, is one that is particularly favorably located, so as to pull a trigger, so to speak, which sets off a very special reaction, one to which the chromatin connections are vulnerable. This reaction, though fairly minutely localized, is not punctiform, but exerts an influence for some distance from its center of origin, and so can result in chromosome breakage.

The crowding together of different chromosomes in the spermatozoan, and their tight looping (probably forming both larger and more minute loops, as noted by Vejdovsky in 1911 in other material), thus gives a good chance for a favorable ionization, that causes one breakage, to cause another breakage nearby. Naturally, two points lying in the same thread not far apart from one another linearly, along this thread, will be more likely to become broken simultaneously in this way, on account of the looping, than points in different chromosomes or far apart on the same chromosome. Thus, the minute rearrangements will be directly proportional in their frequency to the number of favorably placed ions, and hence too to the total number of ions (the dosage) of which the former should form a fairly constant proportion. On the other hand, there will be some breaks derived from a single ionization which are on widely separated points of the chromosome that happened to be close together by accident, owing to the crowding of the chromosomes. That part of the gross rearrangements which had their two breaks caused in the latter way, by single ionizations would vary in frequency as directly as the dosage, while the others, due to independent hits, would vary as the square of the dosage, and so the frequency of all gross rearrangements together would show a relation intermediate between these two types.

On this scheme, then, the breakage is usually a secondary occurrence, not produced by the direct hitting of a gene by an electron. If this is true, it is likely to be true also for gene mutations, as I have urged previously (1932), and so the calculations of gene size which have been persistently offered on the basis of the direct hit idea will have to be discarded. In conclusion, I may say that I think the interpretation of the mechanism of rearrangement offered in the present section cannot yet be regarded as more than a probability, but it is one that offers further opportunity of being tested, in a number of ways.

V. Effects of the Changes in Position on the Functioning of the Genes

From the beginning of the study of chromosome rearrangements in Drosophila, it was noted that they were commonly accompanied by phaenotypic effects like those of gene mutations, affecting the morphology, viability, fertility or other characteristics of the organism. In cases where the first appearance of the rearrangement had been observed, it was found that the phaenotypic change had arisen along with the rearrangement. The first cases of this were spontaneous rearrangements—"Pale translocation" and the inversion of "ClB", but the X-ray work (1928, 1930) soon showed that this was a general rule, no mere coincidence, and that the change acted as though due to a mutation in a gene located at or near to a breakage-point of the rearrangement. The changed gene, in fact, was found to act as an allele of, and often to resemble in its effect, mutant genes of the same locus, when such were known and could be tested in combination with it.

It is now certain that many, if not all, of these changes in gene functioning are not due to intrinsic, autonomous mutations of the genes concerned, but (as in Sturtevant's prior case of bar eye genes that function more actively when in juxtaposition than when in homologous chromosomes) that they are caused by some influence, upon the gene's functioning, of the other genes in its immediate neighborhood in the chromosome. When different genes are substituted for its original neighbor genes, by reason of the occurrence of the rearrangement, the gene in question, now subjected to different influences, reacts differently. Or, if not the gene itself, at least its immediate products, localized in its neighborhood, must be inferred to react differently.

The mere fact that certain genes show changes like mutations in a high proportion of cases when chromosome breakage and reattachment occur near them is not in itself critical evidence that the former changes are due to a position effect, for it might very well have been supposed that these genes are especially liable to undergo mutation when the so-called "bomb" which causes the chromosome break explodes in their neighborhood. But if the genes in question are, like the gene at the locus called "scute" in Drosophila, liable to change in any one of many different ways when they mutate, giving a series of diverse alleles, then there is no reason why a given type of rearrangement, produced by virtue of the topographical accidents of the positions of the different chromosome threads with respect to one another during the process of breakage and reunion, should tend to be associated with a particular qualitative type of "gene mutation" of that gene. But if the effect

on the gene in question is really a position effect, and thus dependent on the type of new gene-associates which that gene acquires, then (as I pointed out in discussing the matter in 1932), a given kind of rearrangement should be associated with a given type of qualitative gene change. Of course it is not likely that exactly the same type of rearrangement should ever be found twice, so as to allow a test of this question, for this would mean that *both* breakage points must be in precisely the same position. However, rearrangements that are very similar might be found, and the qualitative effects accompanying them could then be compared with those accompanying other more widely different rearrangements. This test has been made, and turns out positively.

The test in question was made in connection with an intensive study of changes involving the locus of scute and nearby loci, in which many different cases were analyzed (by a specially devised genetic method which we cannot digress to explain here) in order to determine the precise positions at which the chromosome breakages, if any, had taken place. In more than half the cases demonstrable breakages near the scute locus had occurred, and, as usual, the phaenotypic types of scute were very diverse. Only where the break was to the right of scute was the change in this character marked, however. Among the latter cases, just three were found in which one break was immediately to the right of scute, and in which the second break was in the main chromocentral region of the X chromosome, with the result that the scute gene had been removed from all genes normally to the right of it and had had this region substituted in their place. In all the other cases quite different genes had been placed near scute. Now these three cases were phaenotypically far more like each other than like any of the various other cases. Their resemblance has recently been confirmed by Raffel (1937, in press) in a series of quantitative studies of the bristles in stocks in which, through a long series of crosses, identical genes had been introduced into the major portion of the lengths of all the main chromosomes. In view of the dissimilarities in the other cases, this striking result can represent no mere coincidence, and must mean that the change in the expression of the scute gene was conditioned by its changed position, i.e., by the change in its gene-associations. On the other hand, there were some rather slight, but undoubted differences between these three scutes, even after they had thus been rendered "isogenic", and this again is to have been expected on the basis of the position effect, for the breaks in the "inert" region had not been in precisely the same position in any two of them.

The reverse test, which I also proposed in my former discussion (1932), has now been made too, and it too turns out positively. This is the test of whether, when a given rearranged chromosome undergoes a new rearrangement, of a reverse nature, with consequent restoration of the original or nearly the original order, the apparent gene mutation tends to revert to normal. This has been very nicely demonstrated by Grüneberg, in Haldane's laboratory, with reference to the inversion associated with the mutant character "roughest eye."

An even more direct proof of the position effect has been provided independently by Panchin (1935) in Leningrad, and by Sidorov (1935) in Moscow. Suffice it to say, without going into the technical details, that they have both shown, utilizing different material (the loci of curled and of hairy, respectively), that when there has been an apparent mutation of a given locus, accompanying a rearrangement having one of its points of breakage and reattachment in the neighborhood of this gene yet not so close as to remove all possibility of crossing over with it, then, when an allelic normal gene from a normal chromosome is substituted, by means of crossing over, for the gene which is giving the mutant phaenotypic effect, this normal gene now functions in the abnormal fashion in which the gene previously in that position had functioned, just as though the normal gene itself had now mutated. And, on the other hand, the apparently mutated gene that had been removed by crossing over from the neighborhood of the rearrangement, into a normally arranged chromosome, now gives results quite like those of a normal gene again.

There could be no more conclusive proofs than these of the position effect, and I see no reason to believe otherwise than that the apparent mutation accompanying chromosome rearrangements are, in general (with certain exceptions previously noted as possibilities), in reality the effects of the positional changes. Various studies have shown, moreover, that not only the gene immediately contiguous to a breakage point may be thus affected, but also one somewhat further away, although usually the effect fades out at comparatively small distances. Nevertheless, as a result of it, various genes in the neighborhood of a given one are brought into one functional union, and the various groups of functionally related genes no doubt overlap one another throughout the length of the chromosome. Nevertheless, for any one given effect, such as "scute," the chromosome by no means acts as a whole, since only closely neighboring genes take part in the relationship in question.

It will be seen that, on account of this position effect, chromosomal rearrangements in themselves cause phaenotypic changes. And so, quite apart

from their effect on the recombination of genes in heredity, they will not be completely neutral in respect to natural selection. In perhaps half the cases, however, the changes seem slight, and to these the mechanism termed "drift" by Wright will apply to some extent, in cases like parycentric inversions, in which the productivity of the heterozygote is not reduced. In plants, strange to say, cases of position effect have not yet been discovered, unless a recent finding reported by McClintock be a case in point.

We shall not venture here to discuss the possible causes of the position effect—whether it involves direct chemical influences between genes themselves, that are in this case supposed to be chemically bound together, or local interactions between their products, or influences on their structure coming through the pathway of synaptic forces. As yet, little evidence has been adduced to decide between these very different possibilities.

Before leaving the subject of position effect, it should be pointed out that the chromocentral regions have been found usually to exert an especially marked and far-extending position effect, of peculiar type, resulting in a mosaic expression of the genes that were transplanted to positions in or near to such regions. These so-called "eversporting displacements," do not seem, as various geneticists have thought, usually to involve actual losses, in mosaic patches, of chromosome sections containing the genes concerned. For, as has been argued previously (Muller, 1930), the effect is effect is often only one of degree, and seems capable of reversal, during development, as shown by the arrangement of the spots. Prokofyeva, Belgovsky and I are inclined to attribute the somatic variegation to differences in the mode of formation, or rather in the conformation, of the chromocentral region involved, and these differences probably persist for a number of cell generations, so as to result in whole groups of cells having a common degree of expression of the position effect. In this case, then, the position effect is probably subject to variation correlated with the type of associations between different chromosomes.

On account of this variable but drastic position effect, transplantations of genes from "active" near to chromocentral regions (or *vice versa*) are likely to result in disfunctionings that tend to prevent the evolutionary establishment of such cases of chromosome rearrangement. Hence, normally, we find the chromocentral region confined to the neighborhood of the centromeres and telomeres, even though, in X-rayed material, a part of it very commonly becomes placed in a more or less medial position within a chromosome arm.

It would be impossible to cover, in the given bounds of time and space, all the topics of present interest connected with chromosome rearrangements. We have endeavored here to only touch upon certain of the high spots connected with the problem of the conformation of these rearrangements, their relation to one another, the mechanism of their occurrence, and some of their more immediate effects. We must from consideration here the urgent questions of the relation of rearrangements to so-called gene mutations, which I raised some years ago (1932), whether breaks can occur within genes or merely between them, and whether indeed the so-called "gene mutations are only still minuter variants of the minute rearrangements. The latter question, which both Goldschmidt (1937) and I (1937) have recently raised independently, is now made still more acute by the evidence obtained by Belgovsky, that changes known to be minute rearrangements follow the same law of frequency in relation to dosage of irradiation as gene mutations, and that in this way both alike differ from gross rearrangements. These dosage relations had formed the last distinction positively known to exist between rearrangements and "gene mutations." Together with these questions are bound various others, concerning what the limits of a gene are, the criteria for defining *a* gene, and certain questions concerning the structure and functions of the gene in general. There do, however, seem to be certain possible lines of attack for throwing further light on this series of problems. In fact, this is the genetic field which has been engaging my chief attention for the past five years.

In addition to these, there is a whole series of questions concerning the rôle played by chromosome rearrangements in evolution. It would be interesting to show how much light has been thrown on these by the facts above reviewed concerning the nature of the rearrangements, taken in connection with the known facts concerning their mode of inheritance. We should see that much that has been found in the studies of the comparative cytology of chromosomes becomes clear in the light of these facts, and also that certain rather widely accepted suppositions about the part taken by chromosome change in speciation turn out to be invalid. These latter topics have been treated in another paper, now in press.*

Suffice it in closing, merely to call attention again to the general consistency of the outlook herein presented upon the nature of structural changes in chromosomes with the earlier views of progressive cytologists regarding the persistence of the individuality of chromosome parts, as well as of chromosomes as a whole. We have seen

* "Bearings of the Drosophila Work on Systematics" (in "The Newer Systematics," edited by J. S. Huxley).

(Continued on page 198)

The Collecting Net

A weekly publication devoted to the scientific work at marine biological laboratories.

Edited by Ware Cattell with the assistance of Boris Gorokhoff and Hazel Goodale.

Introducing

Stina Gripenberg, graduate of the University of Helsingfors, Finland, visitor in Woods Hole on an American Association of University Women international fellowship.

Last September Miss Gripenberg left Finland to spend 13 months in this country. She obtained a leave of absence from the Merentutkimuslaitos (Marine Research Institute) in Helsingfors where she is an assistant in the chemical department. She worked together with Dr. Kurt Buch (who has visited Woods Hole several times) at Helsingfors until the latter left; Miss Gripenberg is now the only chemist at the Merentutkimuslaitos.. Her most important study has been on sediments. Of her seven or eight publications, the chief one is a long paper entitled "A Study of the Sediments of the North Baltic and Adjoining Seas." During the past year Miss Gripenberg worked mostly on the west coast—from October to May at the Scripps Institute of Oceanography in La Jolla, California, in June at Seattle and for six weeks this summer at Friday Harbor. In La Jolla her investigations centered on organic matter in sediments and the method of determining carbon by combustion in the simplified way.—*M. F. M.*

EMBRYONIC DEVELOPMENT AND INDUCTION. By Hans Spemann. Yale University Press. 1938. pp. 401.

The papers of Spemann and Mangold have so long trapped embryology graduate students in a maze of subordinate clauses that the publication of an excellent summary and reasoned criticism in the English language will be welcomed by all.

The work commences—a courtesy omitted by many specialists—with a description of normal amphibian development and a brief historical summary of the researches of the classic experimenters. Spemann then passes to the development of the vertebrate eye, which permits a first analysis of the process of induction, and thus to a discussion of the pattern and potencies of the gastrula. Next "organiser" inductions of embryos are described as a basis for the consideration of the terms "induction", "potency" and "determination", and the role these play in the development of the medullary plate.

The concluding chapters deal with theoretical considerations and the various "field theories" are reviewed rather more fully than is customary. Spemann concludes by expressing his "conviction that the suitable reaction of a germ fragment, endowed with the most diverse potencies, in an embryonic 'field', its behaviour in a definite 'situation', is not a common chemical reaction, but that these processes of development, like all vital processes, are comparable, in the way they are connected, to nothing we know in such a degree as to those vital processes of which we have the most intimate knowledge, viz., the psychical ones."

It may be added that neither the bibliography nor the illustrations leave anything to be desired so that the Trustees of the Silliman Memorial Lectures are once more to be congratulated for having added to the literature of science a volume which was as urgently required as it is admirably produced.—*Peter Gray.*

One hundred and sixty-five biologists had registered for the meeting of the Genetics Society of America at nine o'clock this morning. 156 geneticists and their friends attended the Naushon Island clambake last evening.

The editorial board of the journal *Growth* met at the Marine Experiment Station of the Lakenau Hospital for Research, North Truro, Cape Cod, on August 30 and 31 as guests of Dr. Fredrick L. Hammett, chairman of the council. The latter provided an elaborate shore dinner in Provincetown on Tuesday evening. Dr. John Berrill, managing editor, reported satisfactory progress of the journal during its first eighteen months. In accordance with the announced policy of reducing the charge to authors for reprints (which was $4.00 per page for 200 reprints) as rapidly as financial circumstances permit, the rate is to be fixed for the immediate future at $3.50 per page. It was decided to hold a symposium on growth at North Truro next summer provided necessary funds can be secured. One morning was devoted to a general discussion on the scope of the term "growth" and on the subtopics that might form the subject of symposia.

"Return to Life," a new film from Loyalist Spain was presented at the Woods Hole Community Hall, Wednesday, August 31, in two showings in a drive to collect funds for the American Relief Ship to Spain. An audience of 400 saw the film and were greatly impressed by its beautiful photography and its stirring presentation of what is being done in Republican Spain to rehabilitated soldiers wounded at the front. At each showing Dr. Amberson made an appeal for contributions and spoke on the significance of the film and its relation to the general work of filling the American Relief Ship by the Medical Bureau an dNorth American Committee to Aid Spanish Democracy. The proceeds from the sale of tickets and contributions amounted to $325.—*A. Sandow.*

SOME CYTOLOGICAL STUDIES ON VIRUS INFECTED CELLS

DR. ALFRED M. LUCAS
Associate Professor of Zoology, Iowa State College

Fox encephalitis injected into the fourth ventricle produces intranuclear inclusions in cells in various parts of the body. Particular attention was given to the non-nucleolate cells of the anterior medullary velum. Even in the youngest stages the inclusion bodies are Feulgen positive. When the inclusion body first appears it causes the uniformly distributed chromatin granules to aggregate into clumps and to migrate toward the nuclear membrane. This reaction continues until the basichromatin forms a thin shell against the membrane. During early stages of margination the oxychromatin separates from the basichromatin and migrates toward the inclusion body to which it becomes adherent. The oxychromatin is responsible for acidophilic qualities of the inclusion body in this particular case.

The completely marginated chromatin undergoes degenerative changes similar to those of nuclear autolysis. The chromatin breaks up into granules of uniform size and these aggregate into larger clumps. Between these clumps the nuclear membrane is bare. Then follows a loss of nuclear sap which causes shrinkage of the nuclear membrane against the inclusion body, a reaction characteristic of late stages in nuclear autolysis. The only cytological change evident in the inclusion body itself is increasing vacuolization with age.

The series of changes associated with inclusion formation following the action of submaxillary gland virus on guinea pigs is different than the process described for fox encephalitis virus although both inclusions belong to Cowdry's type B. Early stages in inclusion formation in the monocytes are quite similar to those described for fox encephalitis up until complete margination is attained. From this point autolysis seems to involve liquifaction of the chromatin accompanied by shrinkage of the nuclear membrane. The process is similar to that observed in nuclei of liver cells of normal rats fixed about twenty-four hours after the death of the animal. These autolytic changes do not seem as severe in their effects on the inclusion bodies as on the constituents of the nucleus. After autolysis of the nuclei, inclusion bodies may be found free among the monocytes. Some of these bodies are subsequently phagocytosed by other wandering cells.

Nearly all inclusion bearing monocytes have disappeared by the tenth day after inoculation. About the seventh to tenth day intranuclear inclusion bodies appear for the first time in nuclei of duct cells of the salivary glands. They are Feulgen negative but later become moderately Feulgen positive. The reactions of the chromatin in the early stages are similar to those of monocytes up to the point of complete margination. It differs in that the process is fairly well synchronized, whereas in the monocytes all stages may be found at any one time. Both oxy- and basichromatin move to the nuclear membrane. The process up to complete margination requires several days. The chromatin does not remain here but small particles migrate back to the surface of the inclusion body. Later these aggregate to larger clumps and when the process is complete there is only a small amount of material left against the nuclear membrane, much of it is oxychromatin. The final state is attained ten to fourteen days after the inclusions first appear, which is twenty-one to twenty-four days after the virus was inoculated. When this condition is attained it apparently persists for a long period without further change. Autolysis, when it does occur, is similar to that in the monocytes, namely liquifaction of the chromatin and shrinkage of the membrane. The cytoplasmic inclusions composed of minute particles at the limit of microscopic visibility are Feulgen positive. They first appear when the nuclear changes are about half completed.

Ultracentrifugation has been tried on herpes inclusions in the rabbit cornea. This is a type A inclusion according to Cowdry. The inclusions are lighter than either nuclear sap or oxy- and basichromatin. In fox encephalitis and in young duct cells of guinea pigs and in monocytes which are all type B inclusions the inclusions are heavier than the nuclear sap and come to rest above the mingled oxy- and basichromatin concentrated at the centrifugal pole.

(This article is based on a seminar report given at the Marine Biological Laboratory on August 16.)

The following papers were presented at the meeting of the Oceanographic staff held on Thursday, September 1, at 8:00 P. M.: Miss Stina Gripenberg, "The Alkalinity and Calcium Content of the Baltic"; E. E. Watson, "Direct Current Measurements."

At the annual meeting of the M. B. L. Tennis Club Dr. R. L. Carpenter was elected president; Dr. Carl C. Speidel, vice-president; Dr. L. H. Schmidt, secretary-treasurer. Drs. Kidder and Krahl were placed on the executive committee.

The students and staff of the Chesapeake Biological Laboratory held a formal dinner on August 12th in Nice Hall in honor of the 47th birthday of the Director, Professor R. V. Truitt. The group presented him with a scroll in recognition of his unceasing efforts and devotion to the cause of conservation in Maryland during the last twenty years.

THE REMAKING OF CHROMOSOMES

(Continued from page 195)

that this principle now extends even to the matter of the bipolarity versus monopolarity of the parts, and that the telomeres as well as the centromeres, and the genes characteristic of the inert region as well as those more often used in genetic experiments constitute genetically distinctive structures, having definite properties. This is not to say that, by some sort of peculiar mutation, a unipolar gene might not become bipolar, or *vice versa*, or that it might not even acquire such a composition as would render it potentially either the one or the other, depending on given conditions outside of it, as is the case in Ascaris; similiar considerations of course apply to the centromere. But there is no reason to suppose that mere breakage, in itself, ordinarily has such an effect, even though the functions of genes are effected by their positions in the gene chain. And in the light of these conceptions, all at present known cases of structural change, in Drosophila at least, acquire a consistent interpretation. Let us, then, while consolidating this position, move on from it to the attack on the other, more remote questions now appearing before us.

(This article is based upon a lecture given at the Marine Biological Laboratory on September 1.)

INVERTEBRATE CLASS NOTES

The strange and unidentified noise heard last Wednesday noon as the invertebrates were eating lunch on the stern and rockbound coast of Hadley Harbor has at last been tracked to its source. It was the chattering of teeth and the knocking of knees as we ate our ham sandwiches and pickles scooped by hand from the big bottle. As the breezes whistled by our wet slacks and we scambled hurriedly for the all too inadequate sweaters our only thought was of how quickly we could get back to our beloved collecting.

After wading around the remainder of the day up to our knees in mud, and stopping now and then to retrieve a shoe inadvertently left behind, we reached the haven of the *Nereis* and the *Winifred* with a sigh of relief. Our troubles were not yet over, however. Several exhausted inverts stretched out comfortably on the stern of the *Winifred* only to be crudely awakened by a wave that dashed completely over them, chilling and soaking them thoroughly. The *Nereis* showed signs of diving straight to the bottom of the sea time after time, but came up smiling though wet.

The Friday trip, in comparison, came off without a hitch. The weather was perfect; the backyards of North Falmouth, the rocks extending into the harbor and the pools of brackish water proved to be most fertile collecting grounds.

There has been a sudden influx of fiddler crabs into the life of the invertebrate class. There was a symphony orchestra of the little beasts walking across the mud flats of Falmouth. We have been crawling under desks in an effort to retrieve experiments that a moment before were sitting peacefully on the desk. Several little black buttons have been found around the laboratory, which proved on closer inspection to be still other Ucas who had parted with all of their appendages due to the inverts' zeal to study autotomy. There have even been rumors of their advent in coat pockets and beds.

Baseball is beginning to be a sore subject with the south side of the laboratory. Due to a few impartial umpires, they lost Saturday's game by the score of five to three.

Bets are being laid as to when the next lobster dinner will be really enjoyed by the invertebrate class again. We delved into the innermost secrets of their lives last weekend and left them while we spent our day of rest elsewhere. Returning Sunday or Monday morning, we exhibited an involuntary olfactory reaction to said lobsters; our eyebrows and noses went up and the lobsters went out!—*Elizabeth L. Jordan.*

ADDITIONAL PAPERS PRESENTED AT GENERAL SCIENTIFIC MEETING OF THE MARINE BIOLOGICAL LABORATORY

Tuesday, August 30, Morning Session, 9:00 A. M.

Robert Chambers: Cytoplasmic inclusions and matrix of the Arbacia egg.

M. J. Kopac: The Devaux effect at oil-protoplasms interfaces.

M. H. Jacobs and A. K. Parpart: Further studies on the permeability of the erythrocyte to ammonium salts.

Dwight L. Hopkins: The mechanism for the control of the intake and the output of water by the vacuoles in the marine amoeba, *Flabellula mira* Schaeffer.

Tuesday, August 30, Afternoon Session, 2:00 P. M.

E. L. Chambers: The resistance of fertilized Arbacia eggs to immersion in KCl and NaCl solutions.

ALBERT E. NAVEZ: Indolphenoloxidase in Arbacia eggs and the Nadi reaction.

Tuesday, August 30, Evening Session, 8:00 P. M.

CARL C. SPEIDEL: Motion picture showing microscopic changes in fibers of cardiac and skeletal muscle of Invertebrates and Vertebrates during contraction, retraction, and clotting.

W. R. DURYEE: The action of direct currents on the cell nucleus.

W. R. DURYEE: Hydration and dehydration of follicle cell nuclei.

Wednesday, August 31, Morning Session, 9:00 A. M.

ROBERTS RUGH: The effect of the sex stimulation factor of the anterior pituitary on the bullfrog testis.

J. PAUL VISSCHER: Studies on barnacle larvae.

GRACE TOWNSEND: The spawning reaction of *Nereis limbata* with emphasis upon chemical stimulation.

GRACE TOWNSEND: Physiological assays concerning the nature of fertilizin.

ELBERT C. COLE: A study of the integument of the squid, during staining with methylene blue.

CARL C. SMITH and LOUIS LEVIN: The use of the clam heart as a test object for acetylcholine.

Papers Read by Title

C. A. ANGERER: The effect of electric current on the physical consistency of sea urchin eggs.

ROBERT BALLENTINE: Reducing activity of fertilized and unfertilized Arbacia eggs.

LUDWIG VON BERTALANFFY: Studies on the mechanism of growth in *Planaria maculata.*

C. G. GRAND: Intracellular pH studies on the ova of *Mactra solidissima.*

W. R. DURYEE: The action of fixatives on the isolated cell nucleus.

RICHARD W. FOSTER, JOHN D. CRAWFORD and ALBERT E. NAVEZ: Cardiac rhythm in *Pecten irradians.*

M. J. KOPAC: Microestimation of protein adsorption at oil-protoplasm interfaces.

M. J. KOPAC and R. CHAMBERS: Effect of the vitelline membrane on coalescence of Arbacia eggs with oil drops.

A. A. SCHAEFFER: Differences between Scottish and American amebas of the species *Chaos chaos* Müller.

RALPH WICHTERMAN: Does transfer of pronuclei ever occur in conjugation of *Paramecium candatum*?

E. ALFRED WOLF: Studies in calcification IV: A contribution to the problem of skeletal calcification in the teleost, *Fundulus heteroclitus.*

E. ZWILLING: The effect of regeneration of perisarc removal in *Tubularia crocea.*

Demonstrations

E. R. and ELEANOR LINTON CLARK: (1) Marked macrophages. (2) Arterio-venous anastomoses as observed in the living mammal.

E. C. COLE: (1) Methylene blue preparations of the chromatophores of the squid. (2) A low voltage lamp for general microscope use. (3) Methyl methacrylate as a mounting medium for microscopic preparations.

A. S. LEVENSON: Microscopic sections through head and trunk regions of *Fundulus heteroclitus,* prepared by the Gömöre silver nitrate method for the study of calcification.

A. K. PARPART and S. B. YOUNG: A simple glass electrode system.

ROBERTS RUGH: Urogenital system of the male frog *Rana pipiens,* injected to show the course of Spermatozoa from the seminiferous tubules to the Wolffian ducts.

C. C. SMITH and LOUIS LEVIN: The use of the clam heart as a test object for acetylcholine.

GRACE TOWNSEND: Spawning reactions of male *Nereis limbata* in response to glutathione.

J. P. TURNER: Mitochondria and other inclusions in the ciliate *Tillina canalifera.*

THE 1938 PROGRAM OF THE CHESAPEAKE BIOLOGICAL LABORATORY

DR. CURTIS L. NEWCOMBE
In Charge of Research

The teaching program of the Chesapeake Biological Laboratory has followed along its customary course with a few changes. As in the past, courses in invertebrate zoology, economic zoology and biological problems are offered. Due to unavoidable circumstances, it was necessary to remove temporarily, from the curriculum, the course in Diatoms given by Mr. Paul Conger, research associate of the Carnegie Institution of Washington, and the one in Algae regularly offered by Professor H. C. Bold, of Vanderbilt University. Professor R. R. Kudo of the University of Illinois is in charge of the work in protozoology. It is most satisfying to have this work taken up again after its cessation following the death of Dr. Laura Hintze of Goucher College. Professor Kudo is engaged, also, in the following research projects — (1) Protozoan parasites of the oyster and their effects on the host body, (2) Cnidosporidian fauna of Solomons Island, and (3) free-living protozoan fauna of fresh and salt waters of Solomons Island.

Further studies on the oyster are being conducted by Professor M. C. Old of Ursinus College who is also offering course work in Economic Zoology. For about three years, Dr. Old has been studying the boring sponges of the Chesapeake giving special attention to their classification, reproductive cycle, and, in particular, the boring process of this sponge.

Cultural studies of the oyster, *Ostrea virginica,* the blue crab, *Callinectes sapidus,* and the diamondback terrapin, *Malaclemmys centrata concentrata,* are being continued by the Director, Dr. R. V. Truitt, assisted by Dr. F. J. Warner, Mr. Roy Robertson, Mr. Francis Beaven, and Miss Ellen H. Gray. Dr. Warner is making a study of the development of the central nervous system of the terrapin giving particular attention to the time of the development of the cranial nerve motor nuclei. Studies of the life history of the blue crab are being centered at Lynnhaven Bay near the mouth of the Chesapeake. This work is being investigated by Mr. Robertson, while Miss Gray, with headquarters at the Laboratory, is studying the moulting characteristics of this crab.

Little work has been done on the polychaetous Annelids of the Chesapeake. Taxonomic studies of this group are being initiated this season by Dr. Henry W. Olsen, in charge of biological sciences in the Wilson Teacher's College, Washington, D. C., who also is offering the course work in invertebrate zoology. In the field of botany, Mrs. Gladys Mitchell Lang is engaged in a taxonomic study of the flora of the Solomons Island region and vicinity. Her work is designed to provide the Laboratory with an herbarium that will be as complete as possible for this region.

On account of the extent of pollutants that are added annually to the waters of the Chesapeake, a rather significant portion of the Laboratory's work is devoted to the various aspects of this problem. The present studies are centered in the upper reaches of the Bay more particularly near Baltimore City. The problems have been attacked from a hydrographic viewpoint and an effort is being made to determine the area affected by pollutants as well as the concentrations of the various types of waste that may prove destructive to the flora and fauna of the Bay waters. Dr. R. A. Littleford and Miss Anne Shields, of the regular laboratory staff, are carrying on these studies receiving cooperation from various State Agencies.

It is a matter of rather general interest to obtain an expression of the biological fertility of the Bay waters. In an effort, therefore, to contribute to the general knowledge of the productivity of local waters as well as to determine the various seasons of the year when the various larval organisms "set", certain so-called "seeding on" experiments have been in progress for a period of two years. By means of counts and volumetric determinations made periodically, information is being assembled indicating the rate of growth, time of set and degree of survival with respect to a variety of typical bay forms. These studies are being prosecuted by Mr. James Graham of Washington, D. C.

The hydrographic work of the Laboratory, initiated by the writer in June, 1936, is being continued. Attention is focused on the conditions at the mouth of the Patuxent and near-by Bay waters, while monthly profiles are made in the river to indicate its characteristic gradient. The physical and chemical studies are aimed, in part, to determine the effect of the river waters on the conditions in the nearby Bay area. Numerous tributaries flow into the Chesapeake and it is the nature and magnitude of their contribution that is of general interest. The plankton studies are carried on by Mr. B. B. Shepherd, of this Laboratory, whose main interest lies in the Copepod fauna of the region. Intimately associated with the chemical phases of the work are Messrs. F. M. McNall and J. H. McLain.

As a natural outgrowth of the hydrographic investigations, a very definite oxygen-poor layer at the bottom in the deeper regions of the Bay has been established. Present studies are aimed to show the more detailed character of the horizontal and vertical distributions of these waters and to analyze them for various elements and compounds in an effort to explain the existence of the minimum oxygen condition. This particular problem is being studied by the writer in collaboration with Dr. Andrew G. Lang.

Dr. R. A. Littleford is attempting to evaluate the efficiency of the methods used most commonly in quantitative plankton studies. An effort is being made to establish the range of error for each successive step in the two procedures that are employed here.

Mr. David H. Wallace, fishery biologist of the laboratory, is continuing studies on the biology of the striped bass, *Roccus lineatus.* Problems pertaining to rate of growth, age at maturity and migration are receiving particular attention. Plans are in the making for extending the scope of the fisheries studies in the Bay. Here lie many problems that are in need of elaboration.

It is a pleasure to pass on to the readers of The Collecting Net these few notes concerning the activities and problems that are being pursued at this institution. The Laboratory personnel maintain a continual interest in the work of the several laboratories that is reported by The Collecting Net and recognize the unique service to the biologist that is performed by this journal.

THE EFFINGHAM B. MORRIS BIOLOGICAL FARM

DR. EDMOND J. FARRIS
Fellow in Anatomy; in Charge of Operations, the Wistar Institute of Anatomy and Biology.

In 1928 the Effingham B. Morris Biological Farm of 150 acres was added to the equipment of The Wistar Institute. The farm is located in Bucks County, Pa., just off the Lincoln Highway between Philadelphia and New York, four and one-half miles from Bristol, seven miles from Trenton, eighteen miles from Princeton, twenty-six miles from the Philadelphia laboratories and sixty miles from New York City. It is a part of an original grant from William Penn made in 1690 to an ancestor of the late Mr. Morris.

The farm offers unique research opportunities for the study of opossums and batrachian forms, especially Amblystoma. The opossum colony building includes besides the facilities for the colony itself several large and well lighted laboratory and office rooms for research workers. For research on the opossum there are some advantages at the farm which cannot be rivalled elsewhere. There is located a complete collection of opossum eggs and embryos which is probably the largest collection of marsupial embryological material in the world, most of which was presented by Dr. Carl Hartman. Similar material is completely sectioned and ready for use.

Eighty living adult opossums are maintained in the colony for any sort of physiological or anatomical research. Breeding of the opossum is an assured success so that living embryos and pouch young are obtainable in the spring and summer months.

Unusual facilities are offered for investigators interested in working on amphibian material. The normal spawning time of Amblystoma can be delayed and so regulated that eggs can be secured at any time. A large colony of white and black axolotls is maintained within the Spring House Laboratory, as well as an abundance of other amphibian material. Two protected pools and two open pools have been constructed outdoors for rearing amphibia. A frog house and a building for culturing Daphinia for feeding purposes completes the amphibian section.

There are bred and reared many varieties of rats for investigators. At present a limited number of domesticated and tamed gray rats are available. This strain is most hardy, one hundred per cent fertile and resistant to infections.

The facilities of the several laboratories of the Morris Biological Farm are open to qualified investigators. There are four private laboratory rooms available. These may be secured by arrangment with The Wistar Institute upon request. On the research staff of the Morris Biological Farm are three investigators, while three other investigators and two graduate students have been guests this summer. In addition, five investigators from the School of Veterinary Medicine of the University of Pennsylvania have been given permanent appointments and will occupy an experimental building at the Farm early in September.

Of interest in addition to the research material are three residences, one of which is equipped for the comfort of guests. This beautiful Old Farm House with five spacious guest rooms is open to qualified investigators who may wish to work in the laboratory or spend a few restful weeks in a quiet country home surrounded by a biological atmosphere. Here one may enjoy fresh vegetables, certified guernsey milk, etc. Room and board is reasonable.

The Marine Biological Laboratory workers are always welcome. Further information is available.

SOME PROPERTIES OF LIVING CHROMOSOMES

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The fact that the mature salivary gland chromosomes of Diptera are from 2,000 to 5,000 times as large, by volume, as ordinary somatic chromosomes makes them unique material for the study of the properties of living chromosomes.

The usual method for studying "living" salivary gland chromosomes has been to dissect out the gland in the larval body fluid and cover it with paraffin oil to prevent evaporation. We found, however, that this is a very unreliable technique as it, or indeed any injury to the larva, usually causes marked and rapid changes in the chromosomes. We were faced, then, with the problem of trying to see what the living chromosomes are like, without being able to open up the animal to look at them. It turns out, however, that this problem can be solved quite easily. The larvae of many Diptera are transparent, and we discovered that if the larva is flattened out somewhat between a slide and cover-glass, the salivary gland and chromosomes can be seen very beautifully by looking directly through the body wall. In a

preparation of this kind the chromosomes in the salivary gland can be studied with perfect ease under oil immersion, and we should like to emphasize that here we can be quite sure we are dealing with living chromosomes, since this treatment, if carefully done, does not injure the larva in the least. The fly, Sciara, is a particularly nice animal for this purpose because its salivary gland is really a beautiful object for observation and measurement. There are only two rows of cells, and these are absolutely made to order for quantitative work. The nuclei are perfectly spherical, very uniform in size, and individually recognizable by their positions in the gland.

We studied the living salivary gland chromosomes in four genera of flies. Concerning their structure we wish to point out only one fact, namely, that in the *normal* living condition the chromosomes fill, or nearly fill, the entire volume of the nucleus. This is especially clearly seen in Sciara, which has neither chromocenter nor nucleolus, and was observed *in vitro* by Doyle and Metz. The importance of this point is that by measuring the nuclear volume (which is easily obtained from the diameter), we have at once the volume of the chromosomes.

Since we suspected that the degenerative changes which the salivary gland chromosomes undergo when dissected under paraffin oil might be due to asphyxiation of the cells, we tried asphyxiating the whole larva to see how the living chromosomes reacted. The larva was mounted in a special micro gas-chamber so that the living salivary chromosomes could be continuously observed and measured while the larva was subjected to pure CO_2 or N_2 gas. We found that in from one to three minutes after administration of either gas the larva became quiescent, the pulse rate fell markedly and the salivary gland chromosomes began to shrink. The effect of asphyxiation has several very curious features. In the first place, the measurements show clearly that although the chromosomes shrink greatly, the nuclear volume remains entirely unchanged. In the second place, the chromosomes, in shrinking, ball up into a tightly packed spherical mass in the interior of the nucleus. This is a very different sort of shrinkage from that following acetic acid, in which the chromosomes shrink but keep their positions near the periphery of the nucleus. The significance of the asphyxiation type of shrinkage is that it gives us a very neat and accurate method of measuring the shrinkage of chromosomes, simply by measuring the diameter of the clump.

Next we wondered whether this drastic shrinkage of the chromosomes is reversible, so, replacing the CO_2 or N_2 with air or O_2 we found that in a few minutes the heart resumed beating and the chromosomes gradually swelled until they had completely recovered and again filled the nuclei. Nuclear volume as a whole did not change during the process, and this is a very singular thing, because it indicates that this reversible shrinkage of the chromosomes is entirely an intra-nuclear phenomenon. When the chromosomes shrink they give out a clear fluid into the nucleus, and when they recover and swell up again they apparently re-absorb the same fluid, and they may do this repeatedly. From the quantitative side, the results indicate that the chromosomes may lose reversibly up to about 65 percent of their fluid content during exposure to CO_2.

We have seen that there may be chromosome shrinkage with nuclear swelling, as in acetic acid fixation, and chromosome shrinkage and reswelling without change in total nuclear volume, as in asphyxiation. The question now arises, do the chromosomes and nucleus ever shrink and swell together, so that the chromosomes exchange fluid with or through the cytoplasm? Doyle and Metz showed that this occurs when the cells are subjected to various salt solutions. As noted previously, the larva of Sciara is essentially a sack filled with body fluid, in which the salivary glands are freely suspended. What we did, then, to study this effect in living chromosomes was simply to inject Ringer solutions of various strengths into the larval body cavity, thus making the blood either hypotonic or hypertonic to the glands. Chromosomes and nuclei were measured before and after this treatment. We found that injection of hypertonic solution causes a decrease in chromosome volume of at least 35 per cent, and injection of hypotonic solution a swelling of at least 20 per cent, in both cases the volume of the nucleus changing equally, and there being a gradual recovery back to normal.

The salivary chromosomes are sensitive also to more obscure environmental changes such as mechanical pressure on the cytoplasm. In glands across which a little sliver of glass has been laid, the nuclei in the cells under pressure contain shrunken chromosomes, whereas those outside the pressure area are entirely normal.

In summary, we may attempt to define what we mean by "living" chromosomes. To us, the prime characteristic of living chromosomes seems to be their sensitivity to, and ability to react reversibly to various environmental influences. In protein chemistry the principal sign of the denaturation, or what might be called the "death" of a native protein, is an irreversible loss of solubility. Thus it is with chromosomes—as long as they can react reversibly by taking in or giving out fluid, they may be considered, in the chemical sense at least, alive. Alive, but not necessarily normal, for the shrunken chromosomes seen in nearly asphyxiated larvae are probably as definitely ab-

normal as if they were fixed, because marked changes in the chromosomes are always accompanied by unmistakable signs that the animal as a whole is definitely sick.

We may regard the living salivary gland chromosome, then, as behaving chemically somewhat like a sponge, in that it is capable of taking in and giving out an astonishingly large proportion of its fluid content, without permanent alteration of its fundamental internal structure.

(This article is based on a seminar report given at the Marine Biological Laboratory on August 16.)

CALCIUM AND MAGNESIUM IN RELATION TO LONGEVITY OF EGG CELLS

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A few years ago in a study of hypotonic cytolysis of unfertilized Arbacia egg cells, made together with Professor A. J. Goldforb at this laboratory, we found a decrease in resistance to diluted sea water as the cells became older. Working on the hypothesis that the change was in large part due to a loss of elasticity of the egg membrane, such as might be caused by an accumulation of calcium, I found that lowering calcium in sea water greatly prolonged the life of the egg. Continuing with the egg of the clam, Mactra, this effect was corroborated and it was determined also that high magnesium concentration had a similar action or an additive one when calcium was low.

Nonamesset clams of this season, as opposed to animals from Barnstable last season, did not show the low calcium effect as clearly but both were consistent in the prolonging effect of high magnesium. The inconsistency with calcium is regarded, in view of the antagonism between calcium and magnesium, not as contradictory but as indicating the same phenomenon on a different level of egg condition.

These changes in salt ratios are tentatively interpreted as retarding the breakdown of the germinal vesicle since the normal death of the egg is marked chiefly by spontaneous breakdown of the vesicle.

High calcium also has a beneficial influence on the life of the egg, although it is a comparatively small one. This may possibly be due to a fortification of the hyaline layer of the membrane, or perhaps through decreased permeability, against bacterial disintegration products of other eggs. Sea water in which eggs have aged is extremely toxic to other eggs. Experiments have been made to determine whether living bacteria or disintegration products cause this toxicity. Bacterial counts in the experimental calcium and magnesium solutions show no difference in numbers which can account for the effect on longevity of eggs.

It was pointed out that the unfertilized egg is a favorable place in which to study senescence and death because of a regular series of necrotic changes and because the end point of death is definite.

(This article is based upon a seminar given at the Marine Biological Laboratory on August 16).

A NOTE ON MACTRA EGGS

In response to the request of some of the investigators at the Laboratory after the recent seminar report, I am submitting a brief note on the method I have found useful in obtaining the eggs of the clam, Mactra, and also some other data. The animal is rinsed with tap water and dried. The valves gape slightly in a minute or two and the adductor muscles may then be cut easily with a sharp scalpel. The animal is carefully taken out of its shell and the gills trimmed away. The gonads are located anterior to and below the pedal retractor muscle. Gentle pressure over them towards this muscle causes the discharge of eggs or sperm through the gonopores. The eggs are in the germinal vesicle stage. They are a pink color in mass, by reflected light, and tan by transmitted light under the microscope. The diameter of the egg is about fifty micra, the germinal vesicle thirty micra, and the nucleolus about eleven micra.

After insemination the germinal vesicle breaks down within about ten minutes at 25° C., polar bodies are formed at thirty minutes, the nucleus divides at forty minutes, but the cell does not cleave until fifteen minutes later. The lag between nuclear division and cleavage makes experimentation on the relationship between nuclear division and cytoplasmic cleavage more easily possible than is the case with Echinoderm eggs. Except in extreme old age the germinal vesicle persists in sea water and breaks down only on insemination. This is an advantage for study over an egg like that of Asterias, where the germinal vesicle breaks down quickly in sea water. The surface of the egg seems much more elastic, and tough rather than plastic, when compared with Echinoderm eggs. More complete information on this egg, which does not appear to have been previously used experimentally at Woods Hole, will be given in a paper now in preparation.

POSSIBILITY OF ELECTRICALLY AFFECTING DEVELOPMENTAL PATTERN

(Continued from page 181)

forces as may be concerned with the cataphoretic rearrangement of cell contents, and which might therefore conceivably alter the prospective fate of an individual cell, but with such forces as may exist between cells or groups of cells and thus produce a developmental pattern.

It is, of course, well-known that the differential permeability of living membranes gives rise to a difference in potential between the inner and outer surfaces of a cell, and it is further probable that a "cloud" of ions surrounding a cell may give rise to a field of energy which, since it is not of necessity identical with the field surrounding a neighboring cell, may conceivably exercise a force upon the cell. How far such forces would be sufficiently strong to move so large an object as a cell so great a distance as embryological cells are known to move is very doubtful, but space does not permit of a discussion of this question in this place. It is even more doubtful that groups of cells—as has been postulated in some theories of neurobiotaxis—could affect neighboring groups of cells at a considerable distance from them. It is unnecessary, however, to continue theoretical criticism when there is the possibility of an experimental investigation.

It does not matter how potential differences within an embryo may be produced for we can be quite certain that the primary effect which they will have will be to cause movements of ions through the electrolyte in which these cells are placed and thus give rise to weak currents which must undoubtedly exist as continually varying eddies and flows throughout the embryo. If the potentials and currents involved are the cause, and not the effect, of differentiation of embryonic pattern, it should be possible to pass weak currents through a developing embryo and to distort these current movements in such a manner that the embryonic pattern itself will be distorted. It is also within the bounds of theoretical possibility that such currents might electrendosmotically move some organizing substance; or that galvanotropic responses of cells might cause some re-arrangement.

It would also be possible to affect such fields as might exist by the application of strong static or magnetic fields. But again the writer must plead lack of space for omitting these considerations.

A considerable amount of work has been done in the field of the application of currents to developing animals, but the greater part of this work has been done upon fish or amphibian embryos in water and has resulted, as might very well be expected, in the removal of a sufficiency of electrolytes to precipitate the proteins in the egg. This work has resulted only in a demonstration that electrolytes may be electrendosmotically removed from living eggs and has not contributed anything to the question of electrical control of developmental pattern.

Certain early observers have also recorded a series of allegedly electrical effects, mostly too grotesque to warrant consideration, and two recent workers have applied currents to the chick and have recorded the abnormalities so produced as an electrical effect.

There are certain grave technical difficulties which must be overcome before any useful result can be obtained by the application of currents to chick material. It would, for example, obviously be desirable to employ non-metallic electrodes, but these are only feasible where the total experiments do not run beyond a full dozen eggs. The writer has tried liquid bridges, egg-white bridges, agar bridges, and corn starch bridges, but has been forced to abandon them as impractical, and it is now quite evident that if a significant number of eggs are to be utilized it is necessary to employ metallic electrodes. Under these circumstances it is desirable to employ several kinds of electrode so that electrode effects may be detectable by comparison. The first type employed was a "blacked" platinum loop with a ball of agar surrounding the loop. For the second type, a basis of "200-mesh" phosphor-bronze gauze was taken, and this was plated with cobalt, cadmium, silver, silver chloride, palladium and rhodium in an endeavor to find a biologically and physically suitable type; of these, rhodium plated on silver is alone suitable.

The first series of experiments were run with ball electrodes, and, since figures of the results cannot be reproduced, it will be sufficient to say that 342 eggs demonstrated conclusively that such abnormalities as were produced were not in any way correlated either with the current densities employed or with the total quantity of current passed. The abnormalities produced in the largest number were chicks with enlarged hearts, but there were also a type with a reduced central nervous system, a few omphalocephalous types, and a considerable number of blastoderms in which the central nervous system was reduced to a few irregular lumps of tissue with behind them paired pulsating vesicles representing the heart; there were also some laterally expanded types which were at first thought to demonstrate a galvanotropic effect.

It is useless to speak of percentages in dealing with a question of this kind, for the only constant-

ly controlled factor through the experiments was the current, and since the abnormalities were not correlated with this it is useless to endeavor to correlate them statistically with the vast number of unknowns, including the idiosyncrasies of individual eggs, which must also have been present in the experiment.

Normal eggs were found quite frequently among batches through which a current had been passed and this, coupled with the lack of correlation already mentioned, would seem to indicate that the effects could hardly be attributed to the current flowing.

A similar series of experiments were then run using the plated gauze electrodes described. Since, however, in these electrodes the area of metal compared with the area of agar was somewhat greater, it was possible to employ higher current densities, and with these electrodes it was established that 3 δ was a lethal current density. At the same time it became increasingly apparent that the more care taken in the preparation and insertion of the electrodes, the smaller the number of abnormals produced, and it was decided to run a last series of experiments which would, it was hoped, demonstrate conclusively that the current was not responsible for the effects.

A field of asymmetrical current density was erected between one ball and one plate electrode, each of which had been prepared with great care and had been both pre-incubated in egg white and electrendosmotically cleaned in the same material. Using these electrodes it was found possible to develop a chick normally for more than sixty hours while passing 0.1 MA continuously. In three series of experiments, each using 12 eggs in series, no abnormals whatever were found either in the experimental eggs or in the controls and there was an equal number of deaths, presumably due to trauma, in each. A fourth series showed a few abnormals of the enlarged heart type.

Though it appeared from this that a current did not produce abnormalities—i.e. did not distort developmental pattern—the fact remains that abnormals had been produced and that these abnormals occurred with more frequency in the eggs through which a current had been passed than in the controls. It was therefore fairly obvious that there must have been a transport of heavy metal ions from the electrodes and that these were producing the effect. A series of experiments showed that neither platinum chloride nor rhodium sulphate injected, in sub-lethal doses, into the egg white caused abnormalities. A test was then run to determine whether the minute quantities of lead present in the platinum blacking solution could be the cause of the abnormalities shown in the author's, and in previous writers', experiments. Lead chloride was injected in quantities representing from 200γ to 2,000γ Pb and it became immediately apparent that not only were the enlarged heart and suppressed central nervous system effects due to lead poisoning but that the laterally drawn out embryos referred to were also a lead effect. Since the metals used in plating were shown to be without effect, it was evident that the only likely cause of the abnormalities in the plate electrode series was copper being transported in minute quantities through the plating upon the electrodes. It may be added that if a sufficiency of copper was transported to cause a visible green coloration in the agar coating, as happened occasionally through faulty plating, the embryo failed to develop at all and was in any case removed from the experimental records.

Copper chloride in quantities representing from 200γ to 1000γ of copper was injected and the whole range of abnormalities, save only the laterally drawn out lead effect, were reproduced. The enlarged heart effect was brought about with minute dosages, and increasing quanties of copper led first to a slight reduction in the central nervous system and later to the complete suppression of the central nervous system noted as the most advanced type of abnormal found.

Since, then, the abnormalities found could be accounted for by artifacts, the possibility of which was known to be present in both the writer's and in all previous workers' experiments; and since relatively strong currents could be passed between artifact-free electrodes without producing abnormalities, it appeared fairly certain that the current, as such, was without effect.

There remained only those few abnormalities of the completely suppressed nervous system type which had occurred in the controls. It had been noticed that air bubbles had occasionally been included when resealing the eggs, and it was determined to try whether such air bubbles could, by simple mechanical pressure, lead to the suppression of the embryo. It was found that they not only could, but invariably did.

It may be further pointed out that it is well-known that minute dosages of copper and lead stimulate heart tissues in explants of chick heart tissue and that increasing dosages depress growth.

It appears therefore unlikely that weak currents can have any effect upon development, and an adequate explanation has been provided for such effects as have already been recorded by the author or by others. It seems therefore very improbable that such currents as must be produced by existing potentials can have in any way an effect upon the maintenance of developmental pattern.

(This article is based upon a lecture given at the Marine Biological Laboratory on August 26.)